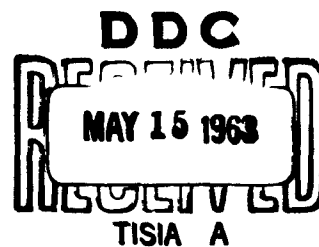CHRONIC WHOLE-BODY GAMMA RADIATION STRESS

IN THE ALBINO RAT AND MOUSE

ANNUAL REPORT

1963

Army Project No. DA-007-49-MD-957

Texas A. & M. Research Foundation

TEES

NG EXPERIMENT STATION

COLLEGE SYSTEM • COLLEGE STATION, TEXAS

CHRONIC WHOLE-BODY GAMMA

RADIATION STRESS IN THE ALBINO RAT AND MOUSE

SIDNEY O. BROWN, Ph. D.
Responsible Investigator

Project No. 188

RADIATION BIOLOGY LABORATORY
TEXAS ENGINEERING EXPERIMENT STATION

and

TEXAS A. & M. RESEARCH FOUNDATION

College Station, Texas

April, 1963

ROSTER OF PARTICIPATING PERSONNEL

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IRRADIATION AND THE HEMATOLOGY OF THE ALBINO RAT

A. Macrofractionated 20 r Per Day Doses of Gamma Irradiation

George M. Krise, Claude J. Coppenger, and Gertrud M. Adam

Earlier studies in this and other laboratories have revealed that the hematologic picture of experimental animals may be altered by whole body exposure to very small macrofractionated doses of ionizing radiation. In the Los Alamos study, reported by Vroman and Anderson (1), people exposed at the rate of 163 mr per week (5 days a week, 8 hours a day) suffered a slight, but definite, decrease in peripheral lymphocytes after several years of exposure. In an earlier report from this laboratory, Krise, Robinson, and Brown (2) failed to note any significant changes in the blood picture of the albino rat when the exposure rate was below 5 r/23-hour day for a total period of one and one half years. Thus, it seems that the rate of exposure to ionizing radiation may play an important role in the manifestation of hematologic damage.

Following a number of experiments on various animal species Blair and others proposed the concepts of permanent and repairable damage. Thus, it was hypothesized that the absorption of ionizing energy produced two sorts of damage, i.e., that which the normal homeostatic mechanism could overcome and repair, and that which remained permanently impaired.

A series of experiments were instituted in this laboratory to study the effects of dose rate and fractionation on the response of the hematologic system of the albino rat. The question to which an answer was sought was "What is the highest possible dose rate that the experimental animal could withstand when given periods of no exposure interspersed with equal periods of exposure?" To this end, this and the several other experiments to be presented were designed and undertaken.

Materials and Methods

Mature adult male Holtzman albino rats were chosen randomly from stock maintained in this laboratory. The average weight of these animals at the beginning of the experiment was 355 grams. The rats had been maintained on Purina Lab Chow and water ad libitum from birth, and this regimen

was followed throughout the experiment.

The 40 animals selected for this study were randomly divided into two groups of 20 animals each. All animals were bled for hematologic analysis on the first day of the experiment. Following this procedure, 20 animals were placed in wire cages, previously described in these reports, and positioned in the irradiation chamber in such a manner as to receive gamma irradiation at the rate of 20 r per 23-hour day. The remaining animals, to serve as unirradiated controls, were placed in similar cages and located in the control room. Both irradiated and control areas share the same air with the temperature maintained at 75± 3 F.

After seven days of exposure the irradiated animals were removed from the chamber and placed in the control environment, and all animals, irradiated and controls, were bled by caudal amputation as described elsewhere and hematological analysis performed. The irradiated animals remained in the control environment for one week, at which time another series of hematological tests was accomplished; then the animals were returned to the same positions in the radiation and control chambers, respectively.

Thus, the irradiated group received 140 r total body dose at a rate of 20 r per 23-hour day every other week for a period of 12 weeks. The total accumulated dose to the whole body as measured in air should have been 840 r. Because of a slight confusion which resulted in the irradiated group remaining three days longer than scheduled in the control environment, the actual accumulated total body dose as measured in air was only 780 r.

Hematological analyses included red blood cell count, white blood cell count, hematocrit, hemoglobin, and differential white blood cell count. Red and white blood cell counts were carried out with a Coulter Counter. The hematocrit was obtained by the micro-capillary method, and the hemoglobin concentration was determined by the cyanmethemoglobin method.

Results

During the 12 weeks' course of this experiment there appeared to be no significant change in the number of red blood cells of the irradiated versus nonirradiated groups (Figure 2). Since this experiment lasted only 12 weeks, the expected gradual decline due to weekly blood loss from experimental bleeding was not evident. However, if reticulocyte counts had been made, it is almost certain, based on past experience, that a stimulation of erythroid production would have been indicated.

-2-

The white blood cell response to these treatments is by far the most important and shall be described in detail. Examination of Figure 3 will reveal that following the first week's exposure there was a dramatic decline in the total white blood cell count. In the irradiated animals this decline averaged 12,000 cells/mm^3. In the c· rols, in accordance with past experience with rats and other experim ⅎl animals, there was an initial decline, the magnitude of whic· less than half that of the irradiated groups.

It has been the experience of the senior author that, unless experimental animals are subjected to experimental bleeding for some period of time prior to initiation of experimental procedures, there is always a change in total white blood cell count, which moves from a high initial value through a stage of steady delcine, to, if the experimental period is of sufficient duration, a steady state. It is suggested that this change in the control animals' white blood cell count represents a state of adaptation of the organism to handling and to the many other changes that might produce initial stimulation of the myeloid elements of the blood and its homeostatic control mechanism.

It is extremely interesting to note in every case but one, the interval of nonirradiation was followed by an increase or recovery of the white blood cell count. This one period was the first period of no stress following the first week of irradiation stress. In this instance the white cell count continued to drop during the nonstress period. In subsequent stress–nonstress periods a recovery–damage sequence was established, with a general picture of a small, but steady, decline in the magnitude of recovery and a steady decline of the total white blood cell count.

These results appear to indicate that the interval of one week is such that complete recovery cannot be accomplished and that the amount of damage to the myeloid regenerative centers is such that, continued over a period of time longer than this experimental period, hematologic failure would result.

When the differential white blood cell counts are examined, it may be seen (Figure 4) that in the group receiving the intermittent irradiation the lymphocyte percent suffers an immediate decline during the first period of irradiation. This initial period is followed by a period of increase, when the percent lymphocytes of the irradiated increases to the level of the control, but then in successive weeks falls to a low which is significantly lower than that of the controls by the 12th week, or at the termination of the experimental period.

In contrast to the lymphocyte response, the percent polymorphonuclear leucocytes (polys) first increase during the initial period of irradiation then decrease to the control value by the fourth week. Subsequently, the polys increase with successive weeks of irradiation exposure until by the end of the experimental period the percent polys is significantly above that of the control.

There appears to be no significant differences between the irradiated and nonirradiated with respect to percent eosinophils, monocytes or stabs.

The hematocrit of both the control and irradiated groups parallel the red blood count and each other, that is, there was an initial decline during the first two weeks followed by an increase with a steady state plateau until the last two weeks of the experimental period when a marked depression in hematc.... was noted (Fig. 8).

In general the hemoglobin concentration remained almost constant for both the irradiated and control groups during the experimental period. There was a slight depression of the hemoglobin in the irradiated group at the 5th week, but was followed by an increase to the level of the controls.

Conclusion

From this study, it might be said that the myeloid elements are less able to meet the insult of 20 r per day for seven days followed by 7 days of no irradiation than are the erythroid elements of the hematopoietic system. This is not an unexpected finding, since it is generally assumed that the myeloid elements are more sensitive to radiation damage than are the erythroid. It is of interest to note the continued response of the myeloid elements during the period of nonstress. Even though the general trend of the irradiated group is toward a decreased response following each subsequent radiation insult, at the end of twelve weeks it appears that the myeloid regenerative ability is still retained.

From these results, it could be surmised that complete removal from an irradiation environment after exposure to equivalent rates and total dose would lead to complete recovery of the hematopoietic system in a relatively short period.
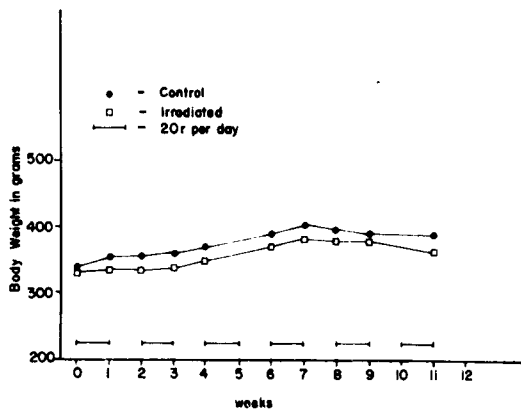
Figure 1. Average weekly weights of control and irradiated male albino rats. The designation (⊢───→) on the bottom of the graph represents the periods of exposure to gamma radiation. Each point represents average of twenty values.
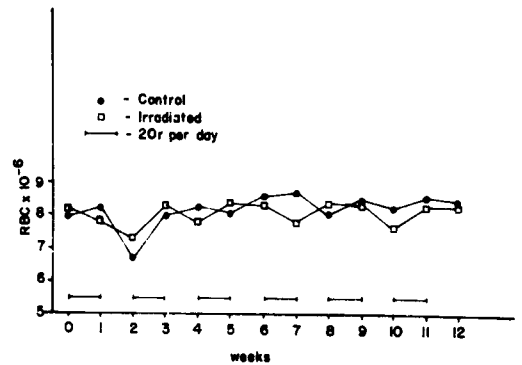
Figure 2. Average weekly red blood cell counts of control and irradiated male albino rats. The designation (⊢───→) on the bottom of the graph represents the periods of exposure to gamma radiation. Each point represents average of twenty values.
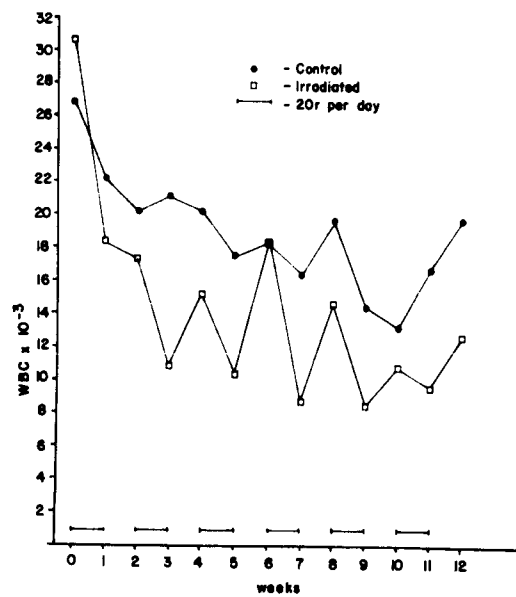
Figure 3. Average weekly white blood cell counts of control and irradiated male albino rats. The designation (⊢───→) on the bottom of the graph represents the periods of exposure to gamma radiation. Each point represents average of twenty values.
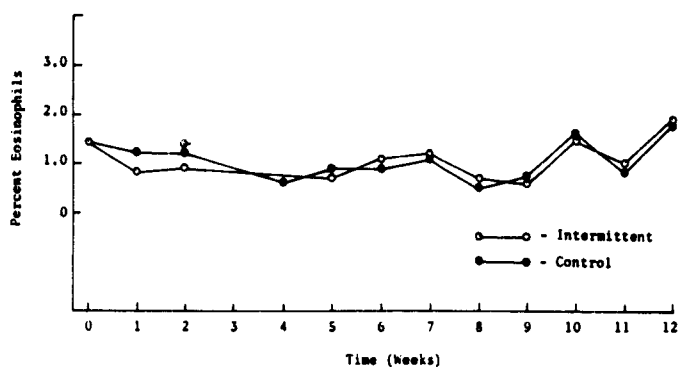
Figure 4. Average Weekly Percent Eosinophils of control and irradiated
male albino rats. Irradiation exposure 20 r/day for a week
beginning with first week, then alternating with a week of
no irradiation. Each point represents an average of twenty
values.
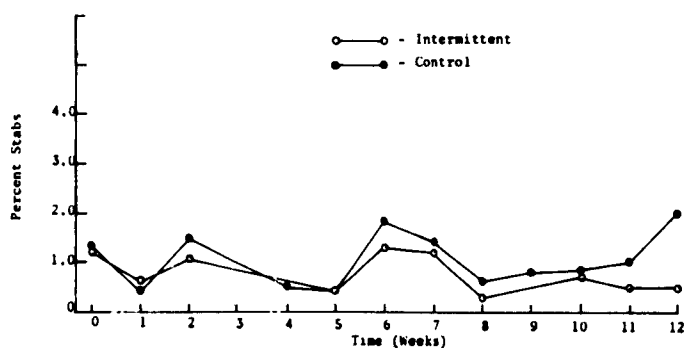
Figure 5. Average Weekly Percent Stabs of control and irradiated male
albino rats. Irradiation exposure 20 r/day for a week
beginning with first week, then alternating with a week of
no irradiation. Each point represents an average of twenty
values.
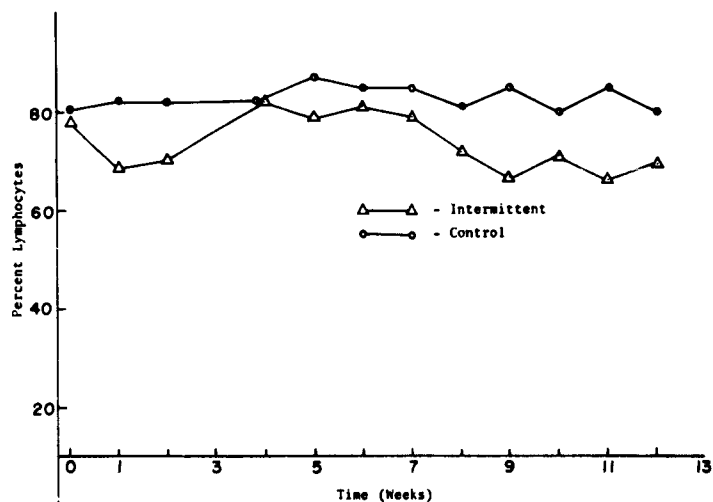
Figure 6. Average Weekly Percent Lymphocytes of control and irradiated
male albino rats. Irradiation exposure 20 r/day for a week
beginning with first week, then alternating with a week of
no irradiation. Each point represents an average of twenty
values.

IRRADIATION AND THE HEMATOLOGY OF THE ALBINO RAT

B. Macrofractionated 40 r per day Doses of Gamma Irradiation

George M. Krise, Paul S. Baur, and Gertrud M. Adam

As previously described in Part I of this series, the intent was to determine the maximum rate of irradiation per day that the hematologic system of the albino rat could tolerate without permanent, nonrecoverable damage. The measure of this damage was the amount of depression of activity of the hematopoietic system as seen from examination of the peripheral blood.

Materials and Methods

In this series 60 male Albino rats of the Holtzman strain, weighing approximately 350 grams, were randomly divided into three groups of twenty each. One group of twenty was placed in a radiation field of 40 r daily and allowed to remain continuously in this field for 11 weeks. The second group of 20 animals were placed in the radiation field at 40 r daily for one week and then removed to the control area for a week. This weekly alternation of radiation and "rest" periods was continued for the eleven-week duration of the experiment. The remaining 20 animals comprised the control group and were maintained in the same type of environment as the irradiated animals, but received only background radiation.

The radiation field in this experiment differed from that used in the 20 r per day study. For this series, the Gamma Facility of the Nuclear Science Center was used. It consists of a nominal 1240 curie source of Co^{60} arranged in such a manner that animals may be placed in small houses in a field for the radiation exposures. These houses are so constructed so as to be portable environment chambers, that is, they can be moved and positioned so that any desired dose rate may be incident to cage positions inside the house; the temperature is controlled within ± 3 F by heaters and air conditioners and the complete insultation of the house walls, roof and floor; the light intensity is constant and a light cycle of 12 hours light, 12 hours dark is maintained on a 6 AM to 6 PM, 6 PM to 6 AM basis. Racks to house animal cages are positioned accurately within the houses by a series

of dosimetric measurements with Landsverk pocket dosimeters so that each cage position receives the same dose ± 10% or less.

Animals are cared for each day during the four-hour period when the source is in the "down", or nonirradiating, position. This means that a radiation day for this facility consists of 20 hours.

The procedures for obtaining blood and carrying out the hematologic analyses were the same as those described in Part I of this series.

Results

For this series, the continuously irradiated group received a total accumulated whole body dose as measured in air in the midline of the cage of 280 r per week or 3,080 r. The intermittent group, those on a week, off a week received a total body dose of 1,680 r (6 exposure periods at 280 r per exposure period).

All animals were weighed each week; the effects of the experimental conditions on the body weights of these animals may be seen in Figure 1. The control animals continued to gain weight until they reached a peak weight of about 410 g at the 8th experimental week. At this time there was an abrupt loss of approximately 10 g. In those animals receiving continuous irradiation at 40 r per day the body weight fell precipitously for the first two weeks of the irradiation period, reaching an average of about 320 grams. After this initial decline, the average body weight of this group increased at about the same rate as did the controls judged from comparison of slopes.

The body weight response of the intermittently irradiated group presents an interesting picture. Following the first week of irradiation the average body weight had fallen about 20 grams, approximately the same loss as that suffered by the continuous group during the first week. During the subsequent week of "rest", i.e. no irradiation, the average body weight of the group rebounded to a level not statistically different from the control group. The second period of irradiation for the intermittent group produced a weight loss almost exactly equal to that following the first exposure period, but because of the compensatory increase during the one week of no irradiation there was a net gain in body weight. This same pattern was evident for the next period of two weeks so that by the end of the third irradiation period or 5th experimental week, the rate of gain of the intermittently exposed group was about the same as the control and continuously irradiated group.

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The white blood cell count in this series offers some very interesting contrasts. The continuously irradiated group suffered an immediate decline in total white blood cell count which averaged 9000 cells per mm^3. This relatively small value was never greatly exceeded during the remainder of the experimental period.

The intermittent exposure group exhibited a rather peculiar response as compared with the results observed in the 20 r per day group described in Part I of this series. Following an initial decline during the first irradiation period there was a very moderate increase during the period of no irradiation. During the second week of irradiation (third week of the experiment), this group experienced a rapid increase in circulating white blood cells, as the count jumped above the value of the first day of the experiment. Subsequent test periods indicated a progressive decline in white blood cells until at the end of the experimental period the white blood cell count of the intermittent closely paralleled the count of those animals continuously irradiated.

In all cases, the irradiated animals exhibited white blood cell counts markedly depressed when compared with nonirradiated controls, even though the controls showed a rather bizarre and seemingly abnormal increase in white blood cell count.

Differential white blood cell counts revealed that both irradiated groups exhibited similar responses with regard to percent lymphocytes and polymorphonuclear leucocytes. The percent lymphocytes initially declined in both groups, with the continuous irradiated group suffering the greatest depression. This depression was maintained throughout the experimental period and was significantly greater than the depression seen in the intermittent group except during the last two weeks of the study.

In considering the polymorphonuclear leucocyte picture, it can be said that the polys' response mirrored the response of the lymphocytes, except that there was a like increase of these cells in both irradiated groups.

In both types of cells, the controls maintained a virtually constant level throughout the course of the experiment.

No striking differences could be noted with respect to the eosinophil percent in any of the three groups. However, in the case of monocytes the intermittently irradiated group demonstrated a striking increase in the number of these cells at three specific periods during the length of the experiment.

Two of these peaks were in phase with the irradiation period, that is, the peak falling immediately after a period of irradiation. However, the third peak of monocyte increase fell at the end of a no irradiation period. Neither the continuously irradiated groups nor the controls demonstrated any remarkable fluctuations in these cells.

Considering immature "polys" or stabs, the group receiving continuous irradiation gave the only significant difference when compared with the controls. By the end of the first week, the number of these cells had increased significantly above that of the controls and remained elevated throughout the experimental period.

Now turning to a consideration of the erythroid series, we may observe that in both the continuously and intermittently irradiated groups, the total red blood cell count fell below the average of the control group by the second week of the experimental period. There was a slight indication of repairative response at weeks 3 and 5 in the intermittent group, however, the general trend was toward a decrease in erythrocyte numbers. At week nine all three groups suffered a decrease in red blood cell counts which continued to fall to the lowest point at the end of the experiment. This decrease was unexpected and not understood, especially in the controls.

The hematocrits were as would have been expected, considering the red blood cell counts, that is, there was a decrease in both irradiated groups with the continuous exhibiting the greatest decline. The unexplainable fall in RBC's was not evidenced in the hematocrit values, casting some doubt on the validity of the RBCC or requiring for explanation a great increase in the average size of the RBC's which is doubtful.

The hemoglobin data support the hematocrit data rather than the RBCC, and there appears to be no significant difference between the hemoglobin value for the three groups, even though there is a suggestion of a lowered hemoglobin for the two irradiated groups as compared to the controls.

Discussion

Since it is planned to compare the three sequences of irradiation dose rates experiments at a later time in this report a discussion of these results will not be presented here.

Figure 1. Average Weekly White Blood Cell Count of control and irradiated
male albino rats. Irradiation delivered at the rate of 40 r
per day. Intermittent group received irradiation for one week
alternating with a week of no irradiation. Each point is an
average of 20 values.

Figure 2. Average Weekly Percent Lymphocytes of control and irradiated
male albino rats. Irradiation delivered at the rate of 40 r
per day. Intermittent group received irradiation for one week
alternating with a week of no irradiation. Each point is an
average of 20 values.

Figure 3. Average Weekly Percent Polymorphonuclear Leucocytes of control
and irradiated male albino rats. Irradiation delivered at the
rate of 40 r per day. Intermittent group received irradiation
for one week alternating with a week of no irradiation. Each
point is an average of 20 values.

Figure 4. Average Weekly Percent Eosinophils of control and irradiated male albino rats. Irradiation delivered at the rate of 40 r per day. Intermittent group received irradiation for one week alternating with a week of no irradiation. Each point is an average of 20 values.

Figure 5. Average Weekly Percent Monocytes of control and irradiated male albino rats. Irradiation delivered at the rate of 40 r per day. Intermittent group received irradiation for one week alternating with a week of no irradiation. Each point is an average of 20 values.

Figure 6. Average Weekly Percent Stabs of control and irradiated male albino rats. Irradiation delivered at the rate of 40 r per day. Intermittent group received irradiation for one week alternating with a week of no irradiation. Each point is an average of 20 values.

Figure 7. Average Weekly Red Blood Cell Count of control and irradiated
male albino rats. Irradiation delivered at the rate of 40 r
per day. Intermittent group received irradiation for one week
alternating with a week of no irradiation. Each point is an
average of 20 values.

Figure 8. Average Weekly Hematocrit of control and irradiated male
albino rats. Irradiation delivered at the rate of 40 r per
day. Intermittent group received irradiation for one week
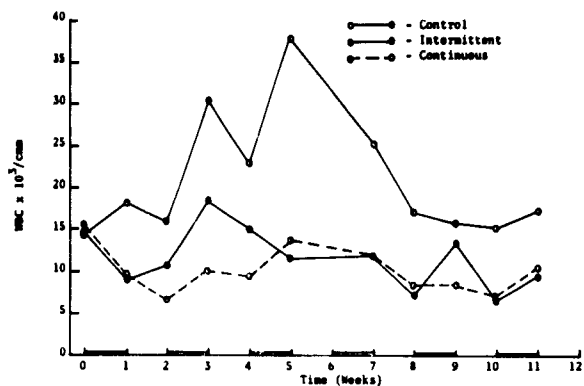alternating with a week of no irradiation. Each point is an
average of 20 values.
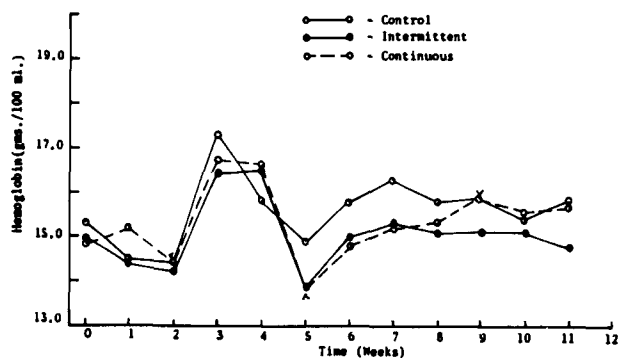
Figure 9. Average Weekly Hemoglobin of control and irradiated male
albino rats. Irradiation delivered at the rate of 40 r per
day. Intermittent group received irradiation for one week
alternating with a week of no irradiation. Each point is an
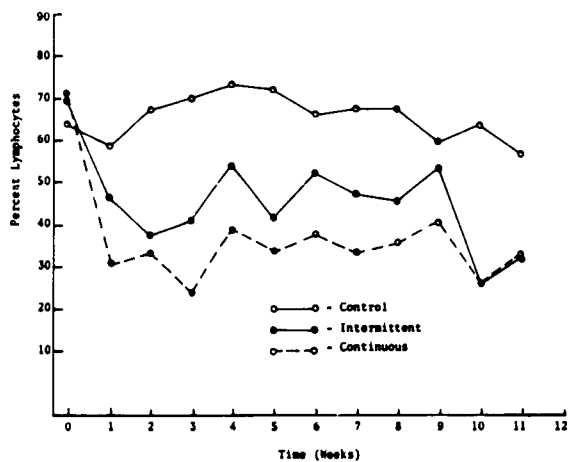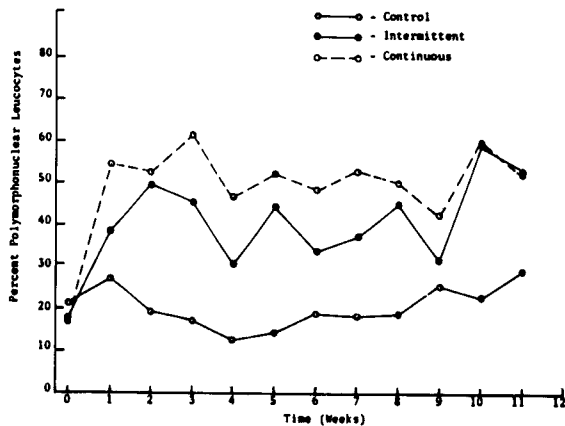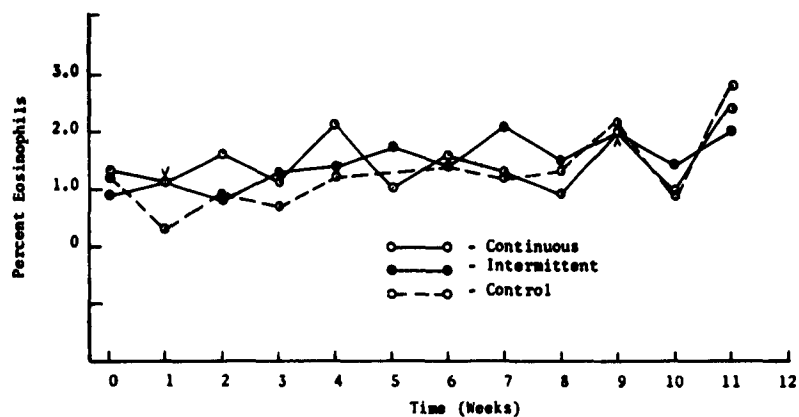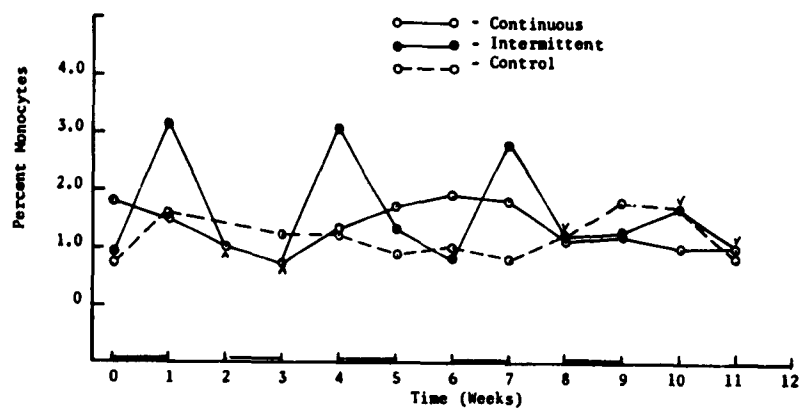average of 20 values.

IRRADIATION AND THE HEMATOLOGY OF THE ALBINO RAT

C. Macrofractionated 50 r Per Day Doses of Gamma Irradiation

George M. Krise, Claude J. Coppenger, and Gertrud M. Adam

This is the third of a series of four experiments to study the effects of daily exposure to gamma radiation at various rates on the hematopoietic system of the albino rat. The previously described experiments have been concerned with doses of 20 r/23-hr. day and 40 r/20-hr. day. The experiment to be discussed here is concerned with exposure of rats to doses of gamma irradiation delivered at the rate of 50 r per 20-hr. day.

Materials and Methods

For this series, 60 male, adult, Holtzman Albino rats averaging about 340 grams in body weight were selected from the colony. These 60 animals were randomly divided into three groups of 20 animals each: one group to be exposed continuously for 11 weeks to gamma irradiation for 20 hours a day; another group of 20 animals to be exposed to an intermittent schedule of one week of irradiation and one week of no exposure for the experimental period; the third group to receive no irradiation and to serve as controls.

Data were gathered concerning the change in body weight and hematologic values under conditions similar to that described in Part II with the exception of the rate of delivery of the gamma irradiation.

Results

It is desirable to first consider the changes in body weight of this series of animals which had been under the experimental condition of food and water ad libitum. In the case of the control group, there was a normal increase in body weight with time which was, except for one point, practically linear indicating a healthy, normal, growing group of animals. The starting weight of this group was about 340 grams and at the end of eleven weeks this group had attained an average body weight of about 440 grams (Figure 1).

The group of twenty rats receiving exposure to continuous radiation at the rate of 50 r per day exhibited an immediate depression in body weight at the end of the first week of treatment. This depression amounted to approximately 10 grams. The weight at the end of one week was maintained for four successive weeks when the animals gained back the weight originally lost during the first week of exposure. Thereafter, each week a gradual but slight increase in weight was recorded until the eighth week, at which time a gradual decline in weight occurred that persisted until the termination of the experiment. At the end of the eleventh week, these animals had a net loss of weight of a few grams as compared to their starting weights. Their average weight at the end of eleven weeks was dramatically lower than the average of the nonirradiated controls (330 as compared to 410 g).

The weight records of the intermittent exposure group reflects the same pattern seen in the body weight data of the 40 r per day animals described in Part II of this series. Following a loss of weight in the first irradiation period, there was an increase during the first period of nonirradiation. This pattern was consistent throughout the experimental period. In each period the amount of damage (measured as weight loss) was offset by a slightly greater increase in the nonirradiation period until the eighth week, following which loss overcame gain so that for the eleven week period there was an over-all net gain of average body weight of approximately 12 grams, as compared to an average net gain of 65 grams in the nonirradiated controls.

During the experimental period, the continuously irradiated group had received a total accumulated dose of 350 r per week or 3850 r while the intermittent group had received 2100 r.

Turning to a consideration of the effects on the myeloid elements of the hematopoietic system, we find that the total white blood cell count in the continuously irradiated group fell from a preirradiation value of 18,000 cells per mm^3 to 5,500 cells per mm^3 at the end of the first seven-day period of irradiation. The largest value ever attained in this group of animals was 7500 cells per mm^3 at the end of eleven weeks.

In the intermittent group, the WBCC fell from an average of 17,520 to 7,000 cells per mm^3 during first week of irradiation. Each successive period of nonirradiation was followed by an increase in WBCC; however, this increase never rose more than 2500 cells per mm^3 over that of the level of the continuously irradiated group, so that at the end of eleven weeks the WBCC for both groups was approximately the same and significantly different from the controls. The control animals maintained a rather constant WBCC with a slight trend to a decrease, the result of conditions considered in Part I of this series.

The differential WBCC presented the expected response to the irradiation treatment. In the continuous group the percent lymphocytes fell sharply during the first two weeks of irradiation. Concomitantly, the polymorphonuclear leucocytes increased in percentage. These trends, decrease of lymphocytes and increase of polys, were maintained throughout the experimental period.

No dramatic or important differences were evidenced in the examination of the eosinophile and "stab" picture. In all groups a similar response to treatment or no-treatment persists. However, in the case of percent monocytes in the continuously irradiated group there is some evidence of a more pronounced response, for in weeks three, seven and nine the percent monocytes for this group were significantly higher than for the controls or the intermittent group.

In the evaluation of erythroid response, it may be seen that there was a separation of the three groups especially after the fourth week of the experiment. At the end of eleven weeks, the control RBCC was somewhat elevated over the count at the start of the experiment, while the intermittent group had suffered a decline early in the experimental period but had gradually returned to normal values. The RBCC for the continuous group indicated that the erythroid elements had sustained more extensive damage, as the number of RBC's at the end of the experiment were some million cells per mm^3 less than at the start.

Hemoglobin and hematocrit values for all group were quite variable with no definite trends except, that there seemed to be separation in the expected order, i.e. control, highest; alternating irradiation, next highest; and continuous, lowest at the end of eleven weeks. The hematocrit values support the data on RBCC.

Discussion

These reults will be considered together with the results of the other two experiments.

Figure 1. Average Weekly Body Weights of control and irradiated male
albino rats. Irradiation was delivered at a rate of 50 r per
day. Intermittent group received irradiation for one week
alternating with a week of no irradiation. Each point is an
average of 20 values.

Figure 2. Average Weekly White Blood Cell Count of control and irradiated
male albino rats. Irradiation was delivered at a rate of 50 r
per day. Intermittent group received irradiation for one week
alternating with a week of no irradiation. Each point is an
average of 20 values.

Figure 3. Average Weekly Percent Lymphocytes of control and irradiated
male albino rats. Irradiation was delivered at a rate of 50 r
per day. Intermittent group received irradiation for one week
alternating with a week of no irradiation. Each point is an
average of 20 values.

Figure 4. Average Weekly Percent Polymorphonuclear Leucocytes of control and irradiated male albino rats. Irradiation was delivered at a rate of 50 r per day. Intermittent group received irradiation for one week alternating with a week of no irradiation. Each point is an average of 20 values.

Figure 5. Average Weekly Percent Eosinophils of control and irradiated male albino rats. Irradiation was delivered at a rate of 50 r per day. Intermittent group received irradiation for one week alternating with a week of no irradiation. Each point is an average of 20 values.

Figure 6. Average Weekly Percent Monocytes of control and irradiated male albino rats. Irradiation was delivered at a rate of 50 r per day. Intermittent group received irradiation for one week alternating with a week of no irradiation. Each point is an average of 20 values.

Figure 7. Average Weekly Percent Stabs of control and irradiated male albino rats. Irradiation was delivered at a rate of 50 r per day. Intermittent group received irradiation for one week alternating with a week of no irradiation. Each point is an average of 20 values.

9.0
8.5
8.0
7.5
7.0
6.5
6.0

RBC x 10⁶/cmm

● ● - Control
● ● - Intermittent
●— —● - Continuous

0   1   2   3   4   5   6   7   8   9   10   11   12

Time (Weeks)

Figure 8.   Average Weekly Red Blood Cell Count of control and irradiated
            male albino rats. Irradiation was delivered at a rate of 50 r
            per day. Intermittent group received irradiation for one week
            alternating with a week of no irradiation. Each point is an
            average of 20 values.

○— —○ - Control
● ● - Intermittent
●— —● - Continuous

44
42
40
38

Hematocrit

0   1   2   3   4   5   6   7   8   9   10   11   12

Time (Weeks)

Figure 9.   Average Weekly Hematocrit of control and irradiated male
            albino rats. Irradiation was delivered at a rate of 50 r
            per day. Intermittent group received irradiation for one
            week alternating with a week of no irradiation. Each point
            is an average of 20 values.

○— —○ - Control
● ● - Intermittent
●— —● - Continuous

19
17
15
13

Hemoglobin (gms./100 ml.)

0   1   2   3   4   5   6   7   8   9   10   11   12
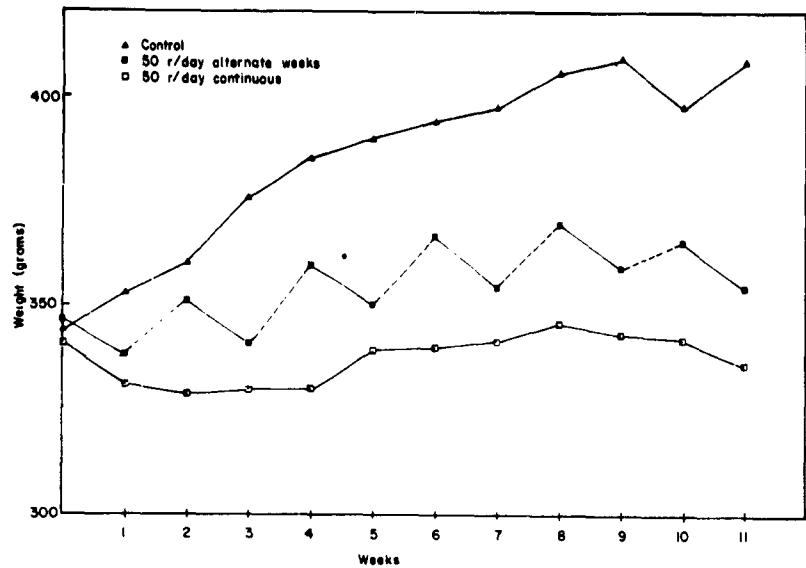
Time (Weeks)

Figure 10. Average Weekly Hemoglobin of control and irradiated male
            albino rats. Irradiation was delivered at a rate of 50 r
            per day. Intermittent group received irradiation for one
            week alternating with a week of no irradiation. Each point
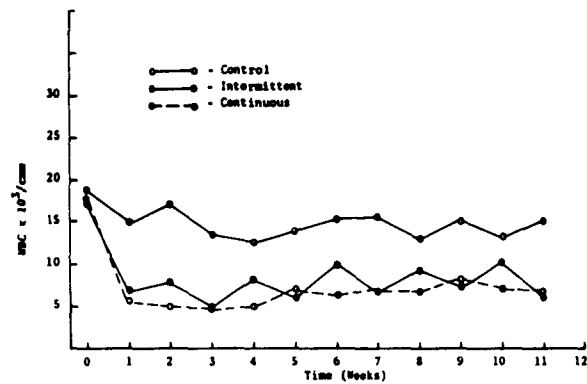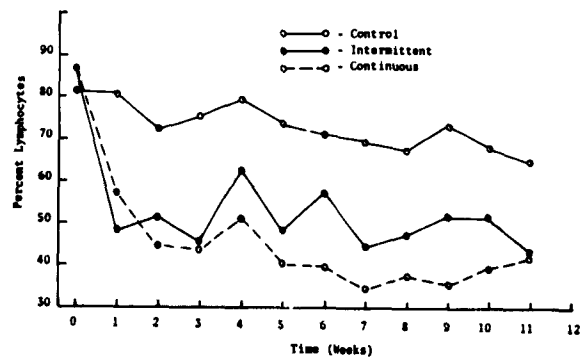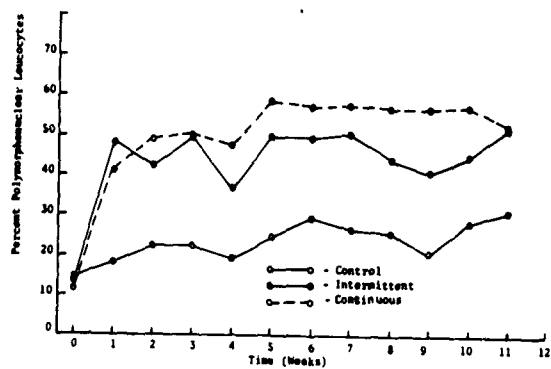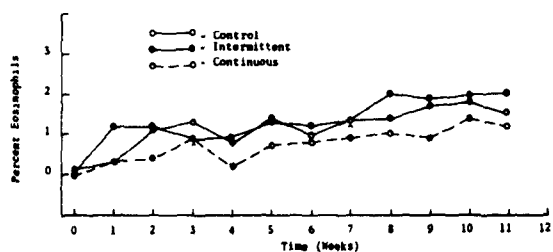   is an average of 20 values.

D. Comparison of Hematological Effects of 20, 40, and 50 r
per day in the Albino Rat

George M. Krise, Claude J. Coppenger, and Gertrud M. Adam

An attempt will be made in this section to compare the effects of 20,
40, and 50 r per day on the hematology of the albino rat. In order to make
these comparisons, it will be necessary to review briefly the irradiation
history.

	Rate r/day	Total Dose (r)
Intermittent Part I	20	840
Intermittent Part II	40	1680
Intermittent Part III	50	2100
Continuous Part II	40	3080
Continuous Part III	50	3850

Body Weights

Considering first the response of albino rats to intermittent weekly
exposures to gamma radiation, it is found that animals exposed to 20 r per
day for seven days followed by 7 days of no irradiation suffer a slight de-
crease in weight gain throughout the 11th week experimental period. However,
there is no indication of an immediate weight loss and no evidence of a de-
crease followed by an increase during the nonirradiation period. This sug-
gests that at 20 r per day insufficient damage occurs to prevent a total weight
gain but is simply enough to produce a retarded but positive growth rate.
This could be interpreted to indicate a decrease in synthetic ability or an
inhibition of mitosis resulting in slower growth. However, as the rate is
increased to 40 and 50 r per day, the damage suffered during the irradiation
period is sufficient to prevent weight gain either through appetite loss or
impairment of the ability to metabolize efficiently the food ingested. If
synthesization were the problem it would seem that the rather dramatic gain
in the nonirradiation period would be prevented. Thus these data suggest
that exposure to chronic irradiation at rates of 40 or 50 r per day produces

inanition in the exposed animals, though this has not been substantiated by food consumption studies.

Next to be considered is a comparison of the body weights of the groups exposed to continuous whole body irradiation for 11 weeks at rates of 40 and 50 r per day. When compared with the intermittent groups at similar rates there appears to be a remarkable difference in effect on weight gain. First, the 40 r per day group, during the first two weeks of exposure, suffered a severe weight loss dropping from an average of about 348 grams to about 322 grams, a total of average loss of about 24 grams. In the group receiving 50 r per day the average immediate loss was only about 12 grams. However, the 50 r group showed only a slight gain through subsequent weeks ending at about the lowest average weight, that is, growth as indicated by weight gains never really got started. In the 40 r group, following the 2nd week of exposure, body weights increased at a rate about equal to the increases for the intermittent and control group but due to the early loss maximum weights at the end of the experimental period were 30 grams that of the controls.

These data could be interpreted to mean that 50 r per 20 hour day produces sufficient damage that the metabolism of the animal (synthetic mechanisms) can just keep up with ensuing damage. It might be expressed in some such fashion that cellular destruction equals new cell formation. However, such a statement needs much experimental evidence to support it. Here again food intake studies could throw some light on the mechanism responsibility for the lack of weight gains in the 50 r group.

Total White Blood Cell Counts

A consideration of the WBCC of the three intermittently irradiated groups, 20, 40, and 50 r per day, suggests a quantitative difference between the lower rate group and the two higher rate groups. In the 20 r group following a rapid and dramatic decline in WBC's there was a response during the periods of no irradiation, the magnitude of which is not seen in the 40 or 50 r group. Also rarely does the WBCC of the 40 or 50 r group approach the values of the control animals while in the 20 r group, during the no irradiation periods, values approached and in one instance equaled that of the controls.

The cyclic response of decrease and increase correlated with irradiation and no irradiation periods is quite apparent in the 20 r group, all but absent in the 40 r group and present but of low magnitude in the 50 r group.

These data suggest that damage during the periods of irradiation is sufficient to prevent or mask any attempt at recovery during the periods of nonirradiation at 40 and 50 r. This is not quite the case, for the response is present but the magnitude of the response is severely depressed.

In the continuously irradiated groups, there is a rather striking difference between those receiving 40 r and those receiving 50 r per day for 11 weeks. Following an immediate first week depression the 40 r group sustained another decline during the second week, while the 50 r group reached a point of maximum depression at the end of the first week. Whereas the 50 r group never seemed to respond to any great extent after the period of maximum depression, the 40 r group seemed to accommodate to the stress, and significant increases in WBCC were recorded for weeks 3, 5, and 9. Since increasing total dose did not appear to alter the WBCC in the 50 r group, one might conclude that the rate of repair just balanced additional insult during the time following the initial period of exposure, that is, the first week. The data suggest that the hematopoietic system of the 50 r per day group has suffered a more permanent effect and one would expect, if the group were removed from the irradiation environment, that the rate of recovery would be slower.

Comparisons of the effects of varying regimen on the differential white blood cell picture would hardly be fruitful. There is a depression in the percent lymphocytes at all dose rates with a suggestion of a rate or total dose dependency. The only significant results, however, are those of the lymphocytes. The other data, percent polymorphonuclear leucocyte, monocyte, and eosinophile, do not lend themselves to meaningful comparisons.

A typical dose-dependent picture is presented by a comparison of the red blood cell count, hemoglobin and hematocrit, that is, maximum effects were noted in the 50 r continuous with less effect in the 50 r intermittent, and less in the 40 r continuous and so forth. In general the magnitude of response of the erythroid elements is less than that of the myeloid elements. The known comparative radioresistance of the erythroid elements of the hematopoietic system as compared with the myeloid is well documented and is substantiated by these results.

II. THE EFFECT OF CONTINUOUS AND DISCONTINUOUS LOW INTENSITY

GAMMA RADIATION IN RESISTANCE TO COLD STRESS IN THE ALBINO RAT

Paul S. Baur, Jr., Sidney O. Brown, and George M. Krise

Introduction

The purpose of this investigation was to study the effect of continuous as well as fractionated radiation in daily doses of 20 r gamma on the resistance of the albino rat to a postirradiation cold stress of 5° C and lower temperatures, applied immediately after the postradiation period. The clinical appearance of the animal, weight changes, oxygen consumption and the gross and histopathological appearance of organs and necrotic lesions were the chief criteria for radiation damage.

The effect of continuous radiation of .8 r daily (0.1 r/hr. for 8 hours) combined with cold temperature was reported by Carlson, Sheyer, and Jackson (1957 a,b). He found that irradiated rats maintained at temperatures of 25° C and 5° showed increased life expectancy in both environmental temperatures; there were no necrotic lesions observed in the cold treatment group.

Following low level chronic irradiation (.84 r/day), testicular damage was noted in animals raised at 5° C, as well as concomitant cases of chronic pyelonephritis and prostatitis. Ear lesions, characterized by dermal inflamatior and epidermal hyperplasia, appeared in all animals. (Griffith, Scheyer, and Lippencott, 1957).

These are the only references available on the combined effects of chronic radiation and cold survival in the albino rat. It is recognized, however, that chronic radiation decreases the leucocyte count and increases the susceptibility of animals to bacterial infection (Miller and Hammond, 1957), to anaphyletic shock (Stoner and Hale, 1958) and secondary tetanus response in mice (1958). Dunaway and Kaye (1961) made observations which indicated a possible decreased cold resistance in the cotton rat (1961). Afrikanova, Vlasova, and Uratkov (1961) observed that hypothermia probably interrupts the pathological effect on the CNS from the irradiated periphery.

In general, it may be said that levels of chronic or low level radiation in the range of 10 to 50 r daily causes a decreased threshold of susceptibility to

environmental stress. It is generally recognized that fractionated doses of radiation, near the sublethal range, decrease the tolerance of irradiated subjects to environmental changes (Bacy and Alexander).

Methods and Procedures

Animal Procedure: In this experiment, 60 male albino rats of the Holtzman strain, weighing approximately 250 grams were utilized. They were divided into three groups of 20 animals each. One group remained continuously in the radiation chamber and was given 20 r/day gamma radiation (23-hour day) for 12 weeks. The second group was treated in identical manner, except at the end of one week the animals were removed from the radiation chamber and allowed to remain in the control chamber for one week. This procedure was repeated for the remainder of the irradiation period. The control group received only background radiation. At the end of the 12-week irradiation period the animals were transferred immediately to a walk-in refrigerator, maintained at 5° C, for a period of 16 weeks. In order to increase the frostbite environment, the ambient temperature was lowered to 2° C during the last week of the experiment. The rats were maintained individually in wire mesh cages to prevent huddling. They were fed Purina Laboratory Chow and the four-day consumption of the individual animal was ascertained through the use of the Fragley feeder system. Body weights were taken at 4-day intervals. Oxygen consumption was determined at the end of the experiment using an electronically recorded pressure reduction technique. The animals were inspected twice daily to determine the time of exitus and the extent of frostbite-induced lesions.

At the termination of the experiment, the animals were given a lethal dose of nembutal and organs were inspected, removed and weighed. Those organs used for histopathological studies were fixed, embedded and stained by standard techniques. Examinations of the organs were made by a veterinary pathologist.

Physical Observations

From Figure 1 it may be noted that during the radiation period a significant weight loss occurred in the irradiated groups, which was most pronounced in the continuously irradiated group. These observations are in accordance with other findings in this laboratory as presented in this report. At the time the animals were placed in the cold chamber, there were no other signs of radiation injury other than decreased size of the testes in the two irradiated groups.

When the irradiated rats were first placed in the chamber, there was a period of adjustment to the environment lasting for 24 to 72 hours. This was characterized by decreased activity, shivering and a weight loss. The weight loss was the most marked during the first four days and this trend continued for twelve days (Figure 2). Following this period, there was a gradual gain in weight, a greatly increased appetite (Figure 3) and accelerated growth of fur resulting in a very dense coat. The first appearance of cold injury in the continuously irradiated group was noted on the 32nd day of cold stress, while no symptoms were observed in the control and fractionatedly irradiated groups until the 40th day. At the end of the sixth week, the most noticeable change was a rigidity of the tail, accompanied by an apparent paralysis and symptoms of chilblain, i.e. hyperemia of the skin, followed by pallor of the superficial regions. These symptoms progressed into those characteristic of frostbite, accompanied by progressive necrosis. The frostbite symptoms, similar to those in the tail, appeared in the ears concomitantly or slightly later. At the end of the sixth week the control animals showed no symptoms, while in the fractionatedly irradiated group they appeared to varying degrees in 55% of the animals. In the continuously irradiated group of animals the symptoms were even more pronounced and occurred in 70% of the animals. Their progression was accelerated, so that in two weeks time 90% of the animals in the continuously irradiated group were affected. In a similar manner the rats in the fractionatedly irradiated group showed a progression of symptoms so at termination 85% of the animals were affected. Changes also occurred in the control groups after the sixth week in the cold chamber, but the symptoms appeared later and were less pronounced. Figure 4 shows the incidence of these symptoms; since animals showing minor as well as major indications of cold injury are included in these numbers, the data presented do not show the severity of manifestations in the irradiated groups.

Determination of oxygen uptake was made two weeks prior to termination and was expressed as cc of oxygen/ minute/cm^2 (body area). Results of this measurement show that there was a substantial difference in the metabolism of both the continuous and fractionated groups as compared to the control animals (See Figure 5).

There was no marked difference in the physical appearance of the two animals of the irradiated groups at termination. Both demonstrated cold injury with the most typical symptoms being necrotic tails, frostbite of the ears and necrotic, prolapsed penis. The same symptoms were apparent in the control group, but less intense in manifestation, and the male reproductive system did not show any symptoms of cold injury.

<u>Histopathological Observations</u>   (by Walter F. Mestanza, DVM)

Group I, receiving continuous irradiation for a period of 12 weeks followed by exposure to a cold environment of 5° C, present the following microscopic findings:

<u>Kidney</u> — Marked eosinophilia of the cortical cells. Hyaline degeneration in drop-like appearance in the convoluted tubules forming hyaline casts of different thickness and degrees of stain. In some animals there is a cloudy swelling of the descending tubules with some glomerular pycnosis.

<u>Spleen</u> — Slight congestion and presence of some hemosiderin. Very mild hyalinization and swelling of the arteriolar capillaries.

<u>Thyroid Gland</u> — The central follicles of the organ are slightly reduced in size and contain a very diluted and poorly stained colloidal material.

<u>Liver</u> — The liver and parathyroid gland present a normal appearance.

<u>Testes</u> — The tubular diameter is considerably reduced in size. Some tubules in the center of the organ appear shrunken and crenated. There is total absence of germinal cells, and only in a few cases there are some remaining spermatozoa. The interstitial cells appear normal in size and quantity. Moderate congestion. Some tubules contain cellular debris and giant cells.

Group II, receiving alternating irradiation with posterior exposure to cold environment, show the following microscopic findings:

<u>Kidneys</u> — Marked fatty infiltration of all the cells lying just immediately under the renal capsule. Isolated, small foci of cellular clumps resting beneath the area of fatty infiltration with slight distortion of the tubular architecture. In some animals there is presence of isolated hyaline material in a few convolute and collecting tubules.

<u>Spleen</u> — Mild congestion. Small hemosiderin deposits and some pycnosis.

<u>Thyroids</u> — Some of the follicles have a columnar epithelium and many of them, although apparently normal in size, contain a light and poorly stained colloid material.

<u>Liver</u> — The liver and parathyroids present no significant abnormalities.

Testes — All the seminiferous tubules are reduced in size, but maintain a normal shape. About 30% of them still contain germinal cells in different degrees of proliferation. Spermatozoa are found only in a few tubules with normal germinal cells. The rest of the tubules show different degrees of degeneration and some of them present giant cells in their lumina. The interstitial cells appear to be normal. In some animals there is a mild intertubular edema.

Group III - Control animals receiving no radiation but subjected to the cold stress. The tissues taken from the control group (III) present no alterations with exception of one animal with a few hyaline casts in the kidney.

Discussion

From the observations made during the course of this experimentation, little doubt remains that both continuous and weekly fractionated low intensity radiation of 20 r decreased the ability of albino rats to withstand continuous cold stress. Since the chief manifestation of this injury occurs in the extremities, it is quite possible the prolonged peripheral vaso-constriction accompanying the adjustment to cold environment would play a major role in this differential response.

Due to vasodilation the rats tail is known to be effective in the thermal exchange between the animal and its environment. Under prolonged cold stress the thermo-regulatory mechanism requires a decreased circulation in the peripheral unprotected appendages. This deprives these structures of blood, thereby making them more susceptible to chilblain, frostbite and necrosis.

The histopathological manifestations described in the accompanying pathology report indicate an involvement of the kidney in the groups receiving the dual stress. The picture of tubular degeneration, fatty and amyloid infiltration, the presence of hyaline casts and glomerular pycnosis was not observed in rats subjected to continuous or fractionated low intensity radiation in previous studies. Therefore, we have here manifestations of the combined stress of low temperature and radiation, and perhaps they may be secondarily due to the extensive necrosis present in the two irradiated series, as compared to the incipient frostbite damage in the control animals.

These observations only serve to indicate a relationship between low intensity chronic radiation insult and decreased adaptability to cold stress.

More extensive investigations are currently under way which will aid in determining more quantitatively   relationship between the two stress factors.

Summary and Conclusions

1. Animals submitted to both continious as well as fractionated irradiation display a definite reduced resistance when later exposed to a low temperature environment. Cold injury experienced in this investigation consisted of frostbite and subsequent necrosis of the appendages with the tail demonstrating the most affliction.

2. Nonirradiated animals are likewise affected by sudden exposure to low temperature, but to a much lesser degree.

3. The major difference between continuously and fractionatedly irradiated animals was the length of time required for cold injury to appear.

4. Loss of circulation in the extremities, due to radiation and cold stress damage, may well be responsible for poor response in the irradiated groups.

TABLE I

BODY - ORGAN WEIGHT RATIOS

(Average)

Animal weight	328.2	347.4	396.7
Adrenal weight			
(Grams)	62.01	61.37	67.93
Percent of body wt.	.01889%	.01767%	.01712%
Liver weight			
(Grams)	12.02	12.32	13.67
Percent of body wt.	3.662%	3.546%	3.445%
Spleen weight			
(Grams)	.6126	.6567	.7277
Percent of body wt.	.1866%	.1890%	.1833%
Prostate			
(Grams)	.4929	.6309	.7940
Percent of body wt.	.1502%	.1816%	.2001%
Sem. Vas.			
(Grams)	.6721	.7244	.7822
Percent of body wt.	.2048%	.2085%	.1971%
Kidney			
(Grams)	2.9742	3.0880	3.3196
Percent of body wt.	.9062%	.8889%	.83666%
Testes			
(Grams)	.8537	.9848	3.3671
Percent of body wt.	.2601%	.2835%	.8488%

TABLE 2

SUMMARY OF DATA

		Controls	Discontinuous Radiation	Continuous Radiation
I. Survival data (20 animals each group)				
	A. Number surviving radiation:	19	20	20
	B. Number surviving cold stress:	18	15	16
	C. Total number of survivors:	18	15	16

Calc. for necrosis excluded animals that died of causes not attributed to cold stress. Actual number of animals receiving cold stress.

		Controls	Discontinuous Radiation	Continuous Radiation
II. Number showing cold injury				
	A. At the 6th week Severe case 6th			
	B. At the end of stress period:			
	1. Frost bite on ears	5	13	13
	2. Tail necrosis	9	13	14
	3. Prolapsed penis	0	2	3
III. Weight data				
	Average weight at initiation	251	246.8	242
	Average weight at end of radiation period	454	402	394
	Average weight at end of cold stress	396	347	328
IV. Food consumption				
	Average weight: High	41.8	40.7	40.4
	Low	21.9	22.2	20.3
V. Oxygen consumption, cc/min/cm^2		.237958	.283077	.2985
VI. Animals that died due to cold stress		05.26	16.66	15.79

DENOTES IRRAD. OF GROUP II

CONTINUOUS
FRACTIONATED
CONTROL

COLD STRESS BODY WEIGHTS

CONTINUOUS
FRACTIONATED
CONTROL

TIME (4 DAY INTERVALS)

DEGREE OF FROSTBITE

ONE WEEK AFTER ONSET ◄── 2 WKS. ──► TERMINAL EVALUATION
OF SYNDROME

OXYGEN UPTAKE
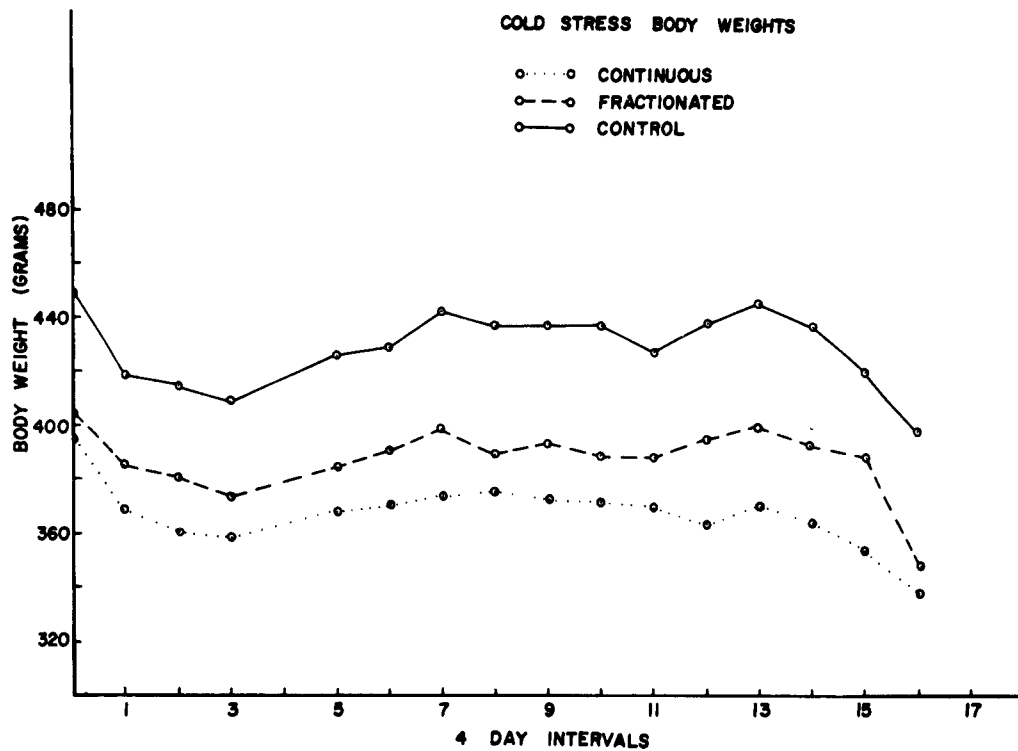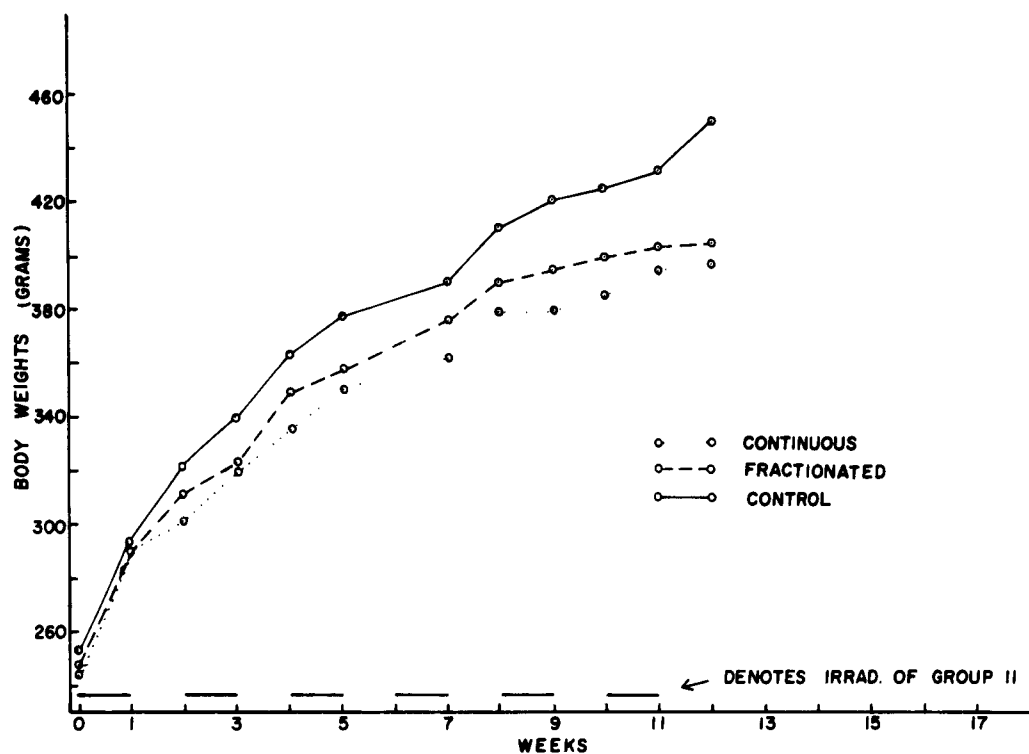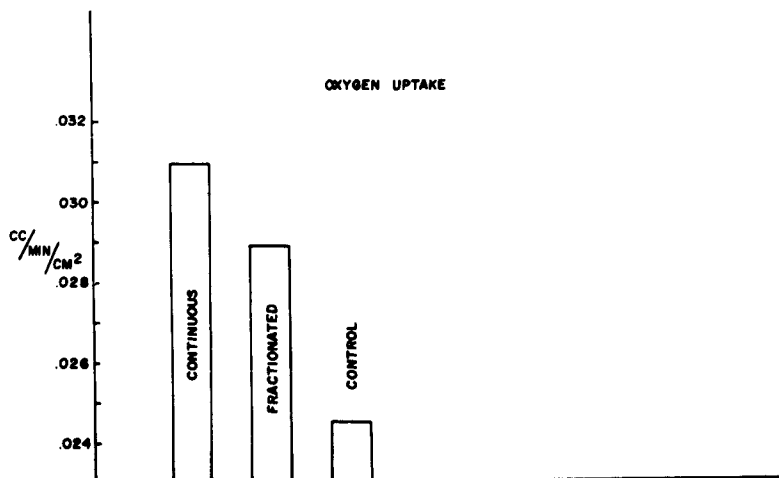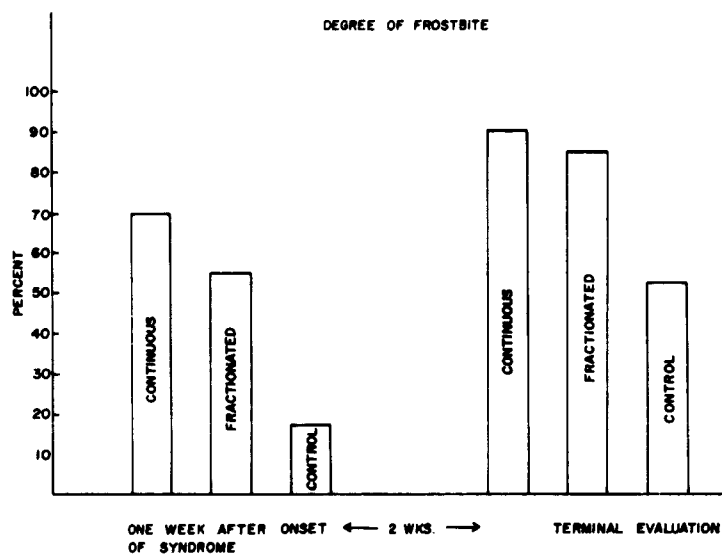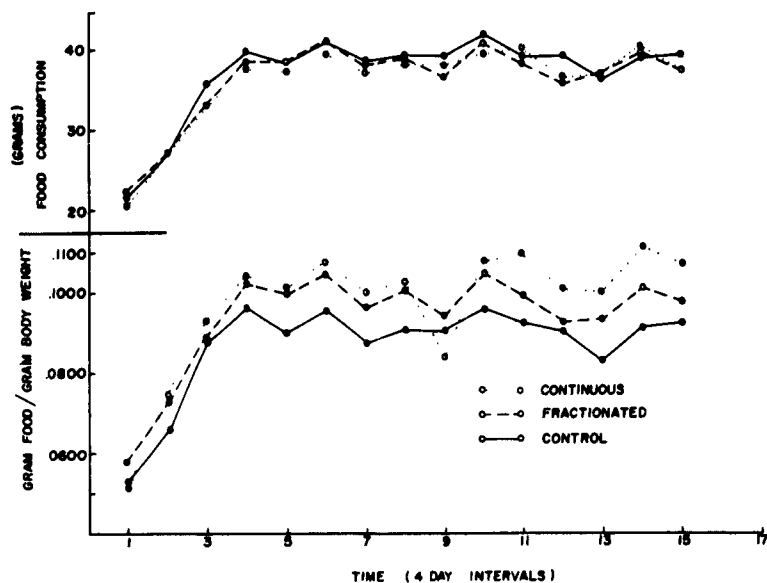
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EFFECT OF CONTINUOUS AND FRACTIONATED LOW INTENSITY GAMMA

RADIATION ON SINGLE DOSE HIGH INTENSITY RADIATION

John A. Hooper and Sidney O. Brown

In this investigation the effect of continuous and weekly fractionated radiation supplied by a Cobalt 60 source on the radiation syndrone produced by 700 r single dose high intensity gamma radiation (523 r/min.) was studied. This investigation was undertaken as a part of a program studying the effect of continuous and fractionated low intensity radiation on the survival of the albino rat when subjected to a secondary environmental stress, which in this case was a single acute dose of gamma radiation near the LD_{50} level.

The effect of multiple small doses of radiation on the survival of laboratory mammals when subjected to a large challenging dose near the LD_{50} level has been the subject of a number of studies. Bloom (1950) reported that mice given 60 r of whole body radiation for 16 days had less cellular debris and more mitosis of the duodenal crypt epithelium after an additional dose of 200 r as contrasted to the controls which received 200 r of whole body radiation only.

After conditioning 100 mice with 144 r X-rays per week of whole body radiation for the total of three weeks, Cronkite and co-workers (1950) exposed them to an acute dose of 703 r. The control mice showed a 41% mortality in 28 days as contrasted to a 26% mortality for the experimental group. No mortalities were obtained in a third group which was given only the conditioning dose. Dacquisto (1959) reports an experiment by Bonet-Maury in which 415 r was given over a period of 55 days in multiple small doses (17 to 72 r). A dose of 800 r was given the mice 16 days after the completion of this "conditioning" dose. The average survival time was 212 as compared to 100 hours for controls.

White mice of the Walter Reed strain were shown by Dacquisto (1959) to develop a definite radioresistance. The $LD_{30(30)}$ is 487 $\pm$ 25.7 r if they receive no radiation, however, if they are given 50 r whole body radiation 10 days prior this figure increases to 560 r $\pm$ 31.0 r; and if the period is 17 days the $LD_{30(30)}$ becomes 617 r $\pm$ 32.0 r. One explanation suggested for this point to hematopoietic stimulation as a possible mechanism along with others.

Vogel and Jordan (AEC Report), however, failed to produce acquired radioresistance in female mice given 50 rads of either Co^{60} or neutron radiation

and later challenged by 320 rad neutron or 900 rads of X-rays. For a more extensive review of the literature see the paper by Dacquisto (1959).

Experimental Procedures

Sixty male albino rats of the Holtzman strain, weighing approximately 150 grams initially were divided into three groups of twenty each. One group of these animals was transferred to the radiation chamber and maintained continuously at 10 r a day (23 hours) for 98 days until the total dose accumulated was 980 r whole body gamma radiation. The second group of animals was placed in the radiation chamber at the 20 r per day level for seven days and, on alternate weeks, removed to the control room for a nonirradiation or "recovery" period. This group likewise received a total dose of 980 r of whole body radiation. The third group maintained in the control at background radiation. During the radiation and subsequent periods all animals were housed individually in wire mesh cages and supplied with Purina Lab Chow and fresh water daily.

At the end of fourteen weeks all animals were given the total of 700 r high intensity radiation (523 r/minute) supplied by a Co^{60} source. This was accomplished by lowering the animal individually into the center of a circular annulus containing the individual pencil sources of Co^{60}. Following the irradiation the animals were returned to a control (nonirradiated) environment, weighed and observed at frequent intervals for symptoms of radiation sickness. The surviving animals are to be necropsied at the termination of this study.

Observations

Weight Change — During the progress of this experiment as may be noted in Figure 1, there was no significant weight changes during the preirradiation or the postirradiation periods. As is generally observed those animals receiving 20 r daily consistently showed on the average less weight gain than the other two groups. The weight did show increased gains during the nonirradiation period as was noted in the studies on the effect of fractionated low intensity radiation on the hematology (see previous report). Following the high intensity gamma dose of 700 r there was no significant changes in the over-all weight trends.

Mortality — At this time 60 days post high intensity, eleven of the thirty rats have died: two deaths occurred in the group given 10 r/day continuous

radiation, four deaths among the animal given 20 r/day fractionated radiation
and five control which had received no radiation prior to the challenge dose
died. The remainder of the animals are alive and no apparent symptoms of
radiation sickness have been noted.

Conclusions

The conclusions that can be drawn at the present time are very limited and
statistically not significant. The loss of 5 animals in the group which was
given no conditioning radiation as compared to the loss of four and two respec-
tively in the other groups receiving continuous and fractionated radiation might
indicate some protective action. However, this conclusion cannot be definitely
drawn on the basis of the data presented in this paper. Another experiment
involving a larger number of animals is under way and should provide conclusive
information on this question.

The negative conclusion that continuous and fractionated radiation do not
predispose the albino rat to mortality or increased radiation sickness following
a sublethal, but high dose of gamma radiation may be much more far reaching
from the military point of view than the radiation protective action indicated
by other workers.

Summary

Three groups of 20 male albino rats each (Holtzmann strain) were subjected
to different levels of exposure to continuous and fractionated low intensity
gamma radiation. One group received 10r/day for 14 weeks, another one 20r/
day alternately with no radiation for the same period of time and the third group
received no radiation. The total accumulated dose for the radiated animals
was 980 r. At the end of 14 weeks all animals were given 700 r gamma radia-
tion at a dose rate of 523 r/min.

Prior exposure to 980 r of accumulated radiation either in low chronic doses
or weekly fractionated doses did not significantly alter the survival time after
a challenge dose of high gamma of 700 r.

All groups given 700 r
at 710 r/min.

o———o   20 r group

△·······△   10 r group

X———X   Controls

+ Denotes weeks in which 20 r animals
were in the chamber.

BODY WEIGHT IN GRAMS

550   450   350   250   150

1   3   5   7   9   11   13   15   17   19   21   23   25   27

Weeks
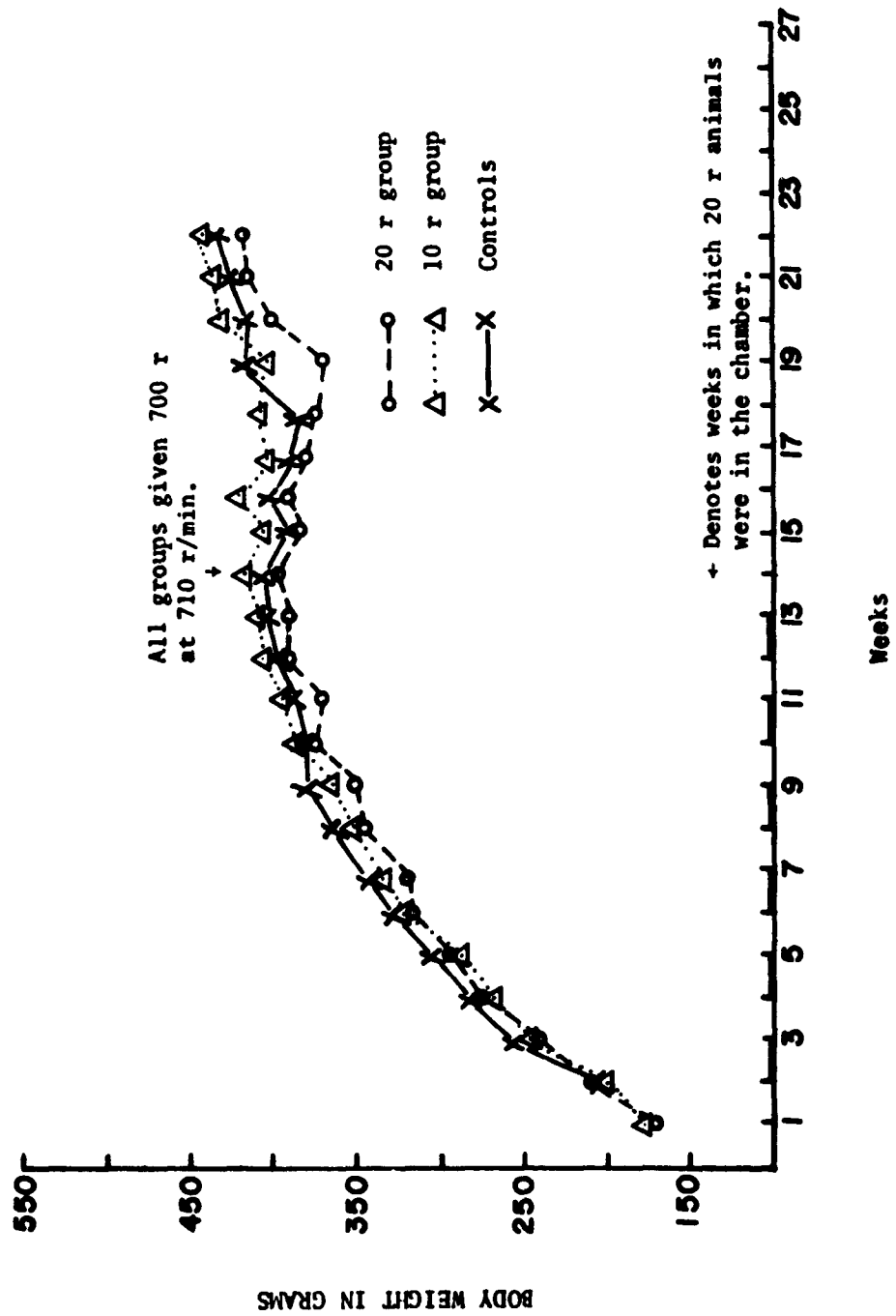
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PROGRESSIVE CHANGES IN THE SEMEN OF ALBINO RATS SUBJECTED TO RADIATION: A TECHNIQUE FOR THE EVALUATION OF RADIATION DAMAGE

Rommon L. Lawson, Roscoe Peterson, Sam B. Barranco, and Sidney O. Brown

This investigation was designed to evaluate the progressively degenerative changes observed in the semen of albino rats as a measure of injury produced by fractionated doses and continued low-dose radiation from a Cobalt 60 source. The criteria to be utilized in this study were the total sperm count, abnormal spermatozoa, presence of foreign cells, sperm volume, and percent motility.

For many years it has been possible to collect semen by electro-ejaculation techniques; for a recent discussion of this problem see Blackshaw (1954) and Marden (1954). By the use of this method (Birnbaum and Hall, 1961) the collection of rat semen and its use in reproduction studies imposes a special problem as the spermia are trapped in an ejaculate which coagulates quickly and firmly into the so-called "copulation plug". This plug, formed under condition of electrical stimulation, blocks the urethra and death by uremic poisoning occurs within a few days. The acid coagulating gland, located adjacent to the seminal vesicles, is responsible for a major part of the congealing activity of the semen following ejaculation. After surgically removing this gland, Scott and Dzuik (1959) showed that semen could be obtained consistently from rats under anaesthesia. Following this lead it was decided to remove the gland and to observe the effects on sequential sperm samples. If this method were worked out the rat could be used to study the effect of various agents on the progressive deterioration of the seminiferous epithelium. Thus, the rat would offer advantages equivalent to those of larger animals.

Many studies (Bacq and Alexander, 1961) show that the seminiferous epithelium is perhaps the most radiosensitive structure in the mammalian body. Boars irradiated with 200 to 400 r Cobalt 60 failed to show evidence of radiation injury on sperm production, motility, morphology or percent of live sperm during the first five weeks following irradiation. Beginning with the sixth week sperm production decreased to a minimum level. After the tenth to the twelfth week postirradiation the sperm production returned to approximately 75 percent of the control level and remained there for the duration of the experiment of 26 weeks (UT-AEC, 1959). In another study (UT-AEC, 1961) rabbits irradiated with 800 r Cobalt 60 gamma irradiation at 49 r per hour showed a decreased sperm count after 45 days of exposure reached a minimum level at 79 days and returned to the control level by 125 days.

Methods of Procedure

A. Surgical Removal of the Coagulating Gland

In order to collect repeated semen samples from the albino rat, a technique for the removal of the coagulating gland was developed. The procedure is as follows:

1. Under Nembutal anaesthesia a mid-line abdominal incision is made to one side of the linea alba, approximately one inch in length.

2. The coagulating gland is stripped from the lateral margins of the seminal vesicles which lie just anterior to the dorsal body wall. Since these glands are not highly vascular and are supplied by numerous small vessels hemorrhage is not a problem; however, a hemostat is applied before finally cutting the gland from its attachment to the base of the seminal vesicle. After approximately one or two minutes the hemostat can be removed and the body wall and skin sutured separately.

3. The animals are allowed a recovery period of 28 days previous to electro-ejaculation tests. The mortality from this operation is less than one percent.

B. Animal Restraint and Electroejaculation Techniques

Without the use of anesthetic the animal is placed into a leather sack-like straight jacket and is held firmly on a collecting board. Using a small animal electroejaculator with a bipolar probe 1/8" in diameter and 3-1/2-4" long, the probe is inserted into the rectum to a depth so as to be in close proximity to the reproductive organs. Properly administered, this mild stimulus to the animal triggers the ejaculatory mechanism causing semen samples to be produced, which in turn are collected in a small test tube for analysis.

C. Procedure for Determining the Function and Effect of the Operation on the Animal

It was decided to first see whether the animals were functional by testing the fertility of the operated males. This was done by mating the males

with two females amd allowing two litters per female to be sired. The same number of unoperated males was used as the control using the same mating procedure.

D. Procedure for Testing High Intensity Fractionated One Dose Radiation on the Seminal Changes in the Male

Before embarking on an experiment to study the effect of low intensity continuous and fractionated radiation, it was decided to test the capacity of a single sublethal 16 hr. irradiation dose of medium intensity to alter the semen of albino rats whose coagulating glands had been removed. After determining the preirradiation characteristics of the semen for three weeks (three tests) prior to irradiation, 40 operated animals were divided into four groups of ten each. The first group was given 50 r (16-hour period), the second 100 r (16 hours) and the third 200 r (16 hours). Semen collections were made at weekly intervals for 14 weeks postirradiation.

The following tests were applied to the semen:

1. The volume of sperm as determine by centrifugation and observing the packed cell volume in the bottom of a conical graduated centrifuge tube.

2. The total sperm count per collection was determined from hemocytometer count on a known volume fraction of the total ejaculate.

3. The percent of motility as ascertained by number of motile and nonmotile sperm within thirty seconds of the time of collection in a microscope field using a warm stage.

4. The percent of living and nonliving sperm was determined by an intravital staining technique employing Fast Green and Eosin in a phosphate buffer pH of 7.3 - 7.4. The sperm are smeared and rapidly dried over a very low flame. This technique is identical with the one used in the examination of the semen of domestic animals for fertility testing.

5. The percent abnormalities were noted. This was done under the highest power optic microscope from the slide in paragraph 4 above. The number of cells showing bent tails, abnormal heads, and other variations from normal were recorded. Each series of the experiment was compared with the controls to rule out abnormalities produced by the technique involved. By fixation and drying in methyl alcohol these slides may be preserved in good condition for future studies.

E. <u>Procedures Used in Testing the Effect of Continuous and Fractionated Low Intensity Radiation Effects on Semen Production in the Albino Rat</u>

Sixty mature male albino rats, from which the coagulating gland had been removed, were tested for three weeks prior to irradiation utilizing the procedures given in D above. When the preirradiation base level for sperm production was ascertained they were divided into six groups of ten animals each and placed in the radiation chamber at levels as follows:

Group I. Control

Group II. 5 r Day Continuous Radiation (23 hours).

Group III. 10 r Day Continuous Radiation.

Group IV. 10 r Day for Alternating Weeks in and out of the Radiation Chamber.

Group V. 20 r Day Continuous Radiation.

Group VI. 20 r Day for Alternating Weeks in and out of the Radiation Chamber.

The same tests, as described in the preceding section, are being utilized to determine the weekly changes in the semen produced by low intensity irradiation.

<u>Results and Observations</u>

A. <u>The Effect of the Operation on the Fertility of the Animals</u>

To see whether the coagulating gland was essential to the fertility of the males, a comparative study employing operated and unoperated males was undertaken. The results of the fertility test of twenty litters each born to the operated and nonoperated males is shown in the table below:

	No. of Litters	No. Born	Alive	Dead	Av. weight of Offspring
Control	20	205	188	160	5.64
Operated	20	209	190	190	5.97

None of the values given above are significant; the removal of the coagulating gland did not influence the fertility of the rat.

B. Observations on the Effect of Single Daily Doses of Medium High
    Intensity Radiation on Semen Production

    1. Effect of Radiation on Weight Changes

    The average body weight of these animals at the initiation of this study
was 480 grams which was not altered to any marked degree by the levels of
radiation administered; however, during the tenth week of the experiment
(the seventh postirradiation week) there was a respiratory infection which was
disseminated throughout the experimental and control groups alike, causing
on the average some 40 grams of weight loss. This infection was not treated
specifically, but it appeared to be self-limited, and the animals regained a
part of their weight during the remainder of the experimental period of sixteen
weeks.

    2. Effect of Radiation on the Packed Cell Volume of the Semen (Fig. 1)

    The readings obtained during the first three weeks of the preirradiation
regimen showed some variation. This was due in part to a change in the
method used to quantitatively determine this factor. By the end of the
third week, we had learned to differentiate between the sperm cell volume
and the volume of other exudates which appeared in the semen; thereafter,
the results were comparable. For this reason, the first two week's readings
should be ignored. After the sixth week (the third week postirradiation) there
was an apparent relationship between the different levels of radiation and the
volume of the sperm cells. The controls almost consistently had a larger
volume, while those animals receiving 200 r showed the smallest packed cell
volume. The groups receiving other levels of radiation were intermediate
between these extremes.

    3. Effect of Radiation Treatment on the Total Number of Spermatozoa (Fig.2)

    There was considerable variation in the total number of spermatozoa per
ejaculation as determined by the method used. Two factors may account for
this, first the inability to exactly duplicate electro-ejaculation stimulus due
to handling of the animals, the placement of the electrodes, and perhaps
other psychic factors difficult to ascertain. In fact, after a few treatments,
some animals became conditioned to ejaculate prematurely by the handling
process without the electrical stimulus.

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The second factor, which is clearly discernable in the controls, was the illness due to the lung infection which is reflected in the total number of spermatozoa beginning on the ninth week in the controls. Again those animals receiving acute doses of 200 r whole body radiation showed the smallest number of spermatozoa. In all groups, there was an apparent "recovery" phenomenon during the latter weeks of the experiment. We cannot say whether this recovery trend was from radiation damage or the pneumonia.

4. Effect of Radiation on the Motility of the Spermatozoa (Fig. 3)

The percent motility observed in the control animals was approximately 50%. This particular criterion was relatively consistent throughout the experiment. The irradiation treatment of 50 and 100 r showed no significant difference from the controls; however, those animals receiving 200 r whole body radiation showed a definite decreased motility when compared to the unirradiated group with a recovery from the radiation damage occurring during the last four weeks of the experiment. It is possible that the percentage of motility may improve with more refinement of technique, in fact, the type of diluent used has been shown to affect the motility to some extent. However, this should not change the overall observed relationship to radiation damage since identical techniques have been used throughout the experiment.

5. Observations on the Sperm Both Normal and Alive as Affected by Radiation (Fig. 4)

In using the highest power (900 x) the rat sperm stained with an intravital method (fast green and eosin) did not show the clear differentiation that is observable in the bovine and other large animal sperm. Therefore, considerable discretion must be exercised in this technique which undoubtedly would to some extent depend on the individual observer. Certainly, some improved techniques must be perfected for the rat spermatozoa. These observations were all made by one person (R. L. L.) and therefore should be consistent throughout this experiment. From Fig. 4 it may be observed that all the animals receiving any radiation showed marked decrease in the percentage of live spermatozoa in the earlier weeks of the preirradiation period. At the eighth week the percent of live sperm dropped, which may have been due to the infection. During the latter phase of this experiment the percentage of the live spermatozoa in the irradiated animal increased to approximately the control level. The percentage of live sperm in the rats receiving the highest irradiation level in general was lower than in the remainder of the radiation groups.

6. Effect of Radiation Treatment on the Percentage of Abnormal
   Spermatozoa (Fig. 5)

The first week following acute radiation the percentage of abnormal sperm
found in the ejaculate of the controls also showed some increase which coin-
cided with the period of infection. There was no significant difference in the
percentages of abnormalities in the sperm of any of the radiation levels; how-
ever, in general those rats receiving 200 r showed the highest percentage. In
the 4th to 10th week (1st to 7th postirradiation) the difference between the
percentage of abnormal sperm in the control and all radiation groups was signi-
ficant.

C. The Effect of Continuous and Weekly Fractionated Low Intensity Gamma
   Radiation on the Changes in the Semen of Albino Rats

The studies of the evaluation of the low intensity, continuous and fractionated
radiation effects through changes in the semen are in progress. The animals have
been on this experiment for 10 weeks and on the radiation regimen for seven weeks.
This experiment is to be carried on for at least eighteen weeks. The results to
date show a relationship of radiation levels to the production of abnormal sperm
and the percentage of motility. The analysis of the data is underway and the
results of the study will be included in a subsequent report.

Discussion and Conclusions

By the use of the techniques given above it is possible to follow the progres-
sive changes in the semen of the albino rat. This technique alone may be of
value in the study of radiation effects as well as the numerous fertility drugs,
the testing of which is now in vogue. By the use of this technique we have
been able to observe the effects of relatively low doses of radiation on the
albino rat. The radiation dose of 50 r delivered over a 16-hour period was
sufficient to significantly alter the number of abnormal spermatozoa and to de-
crease the motility as observed in the weekly samples of sperm obtained by
electro-ejaculation techniques. It is quite possible that with sufficient in-
vestigation the semen, as well as the blood, may be utilized to detect low
level radiation damage.

Summary

A technique has been developed to surgically remove the coagulating gland and to obtain by electro-ejaculation samples of semen sufficient for analysis. The following tests have been carried out: packed cell volume, total number of spermatozoa, determinations of motility, number of living, and abnormal sperm.

This technique was applied to groups of ten albino rats each, receiving 0, 50, 100, 200 r of whole body radiation over a 16-hour period. All of the criteria used indicated to some extent radiation damage with the sperm motility, dead sperm, and abnormal sperm measures being the most influenced by the different levels of radiation.

At the present time, an experiment is in progress involving the seminal changes in rats receiving continuous and weekly fractionated doses of radiation.

Figure 1. Average counts of sperm motility in all radiated and control groups

Figure 2. Alive and normal sperm numbers in all radiated and control groups

SPERMATOZOA MOTILITY

ALIVE & NORMAL SPERMATOZOA
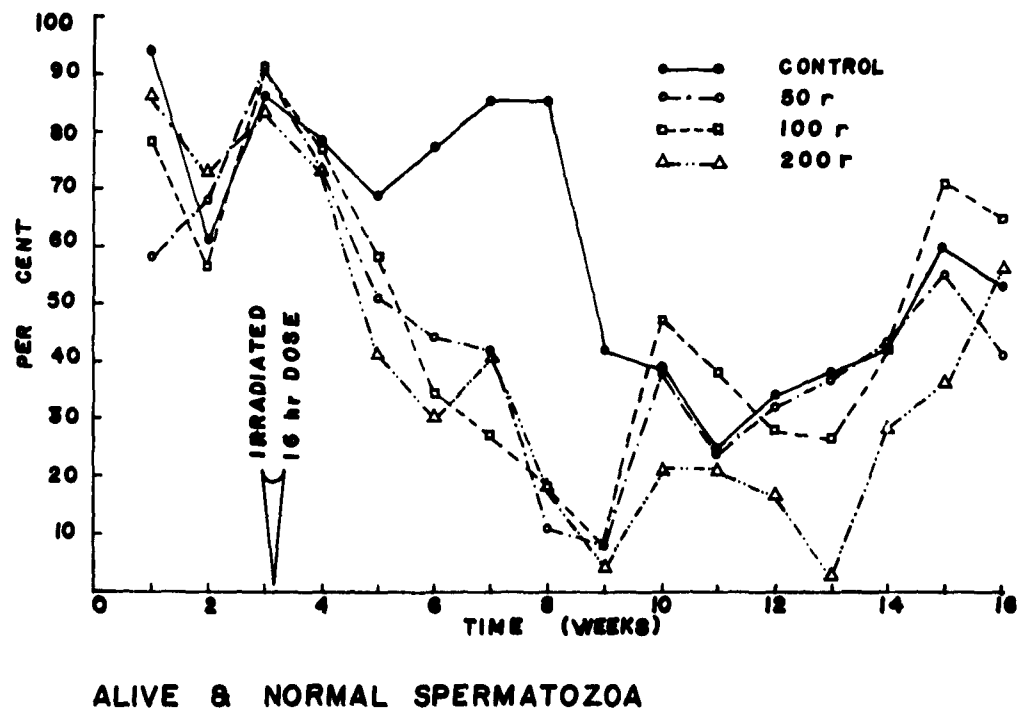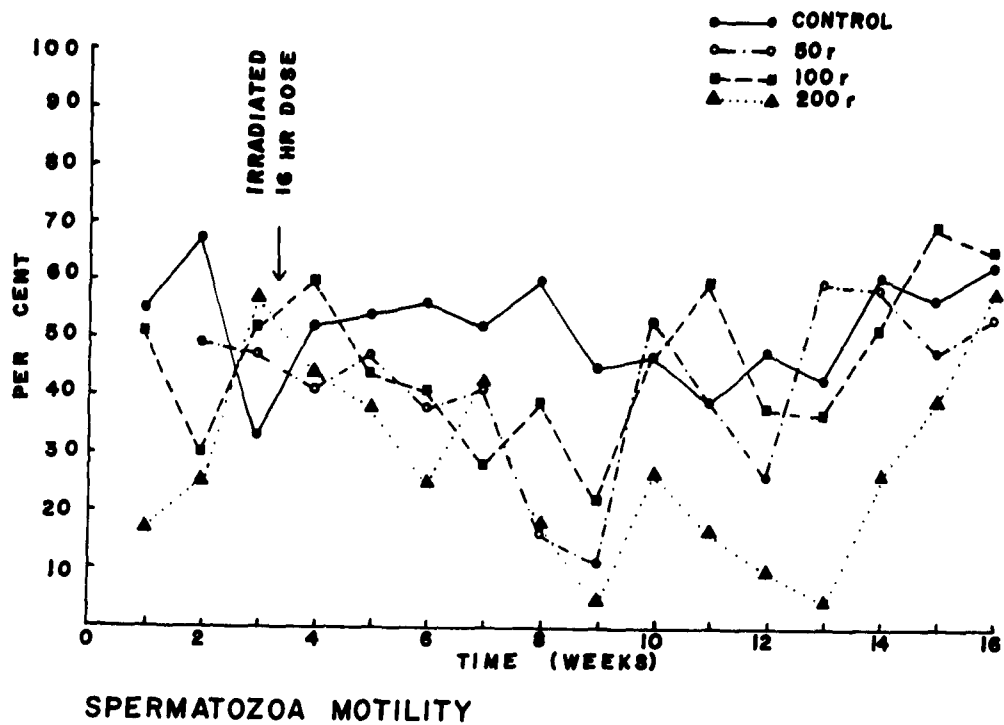
Figure 3. Total packed cell volume in all irradiated groups and
the control group

Figure 4. Total sperm counts in all experimental groups
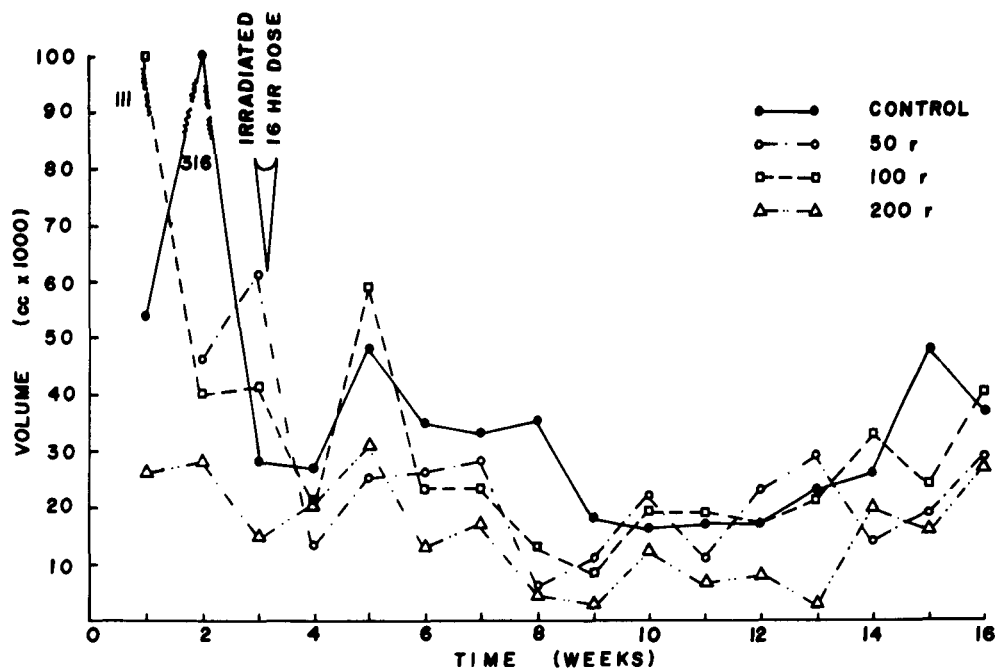
TOTAL P.C. VOLUME
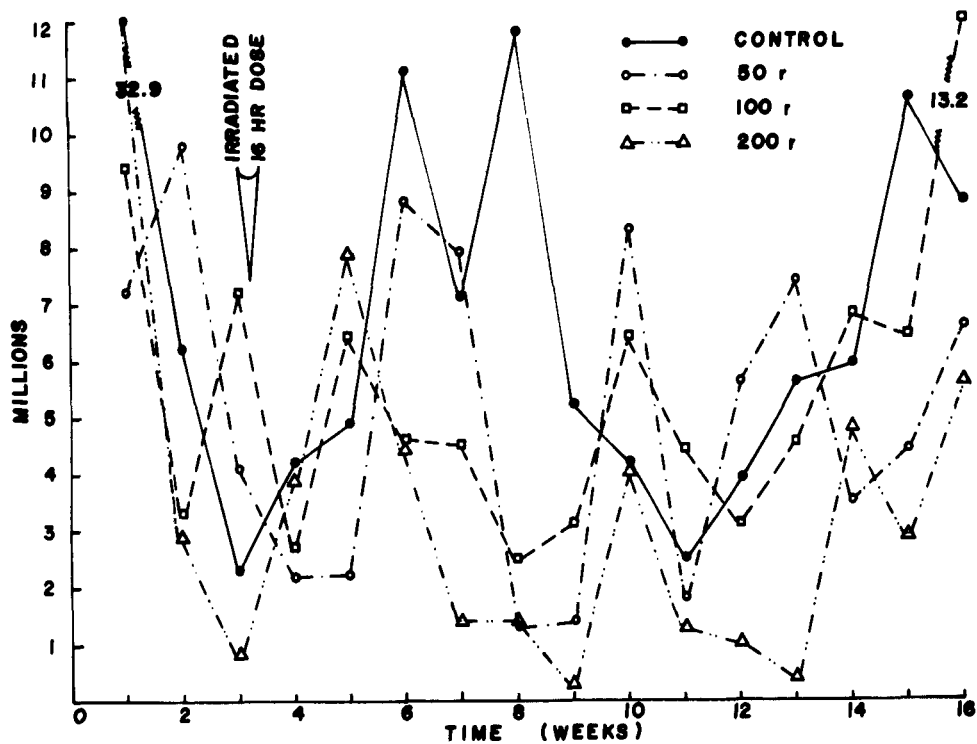
TOTAL SPERMATOZOA

Figure 5. Incidence of abnormal spermatozoa in all irradiated
groups and the control group
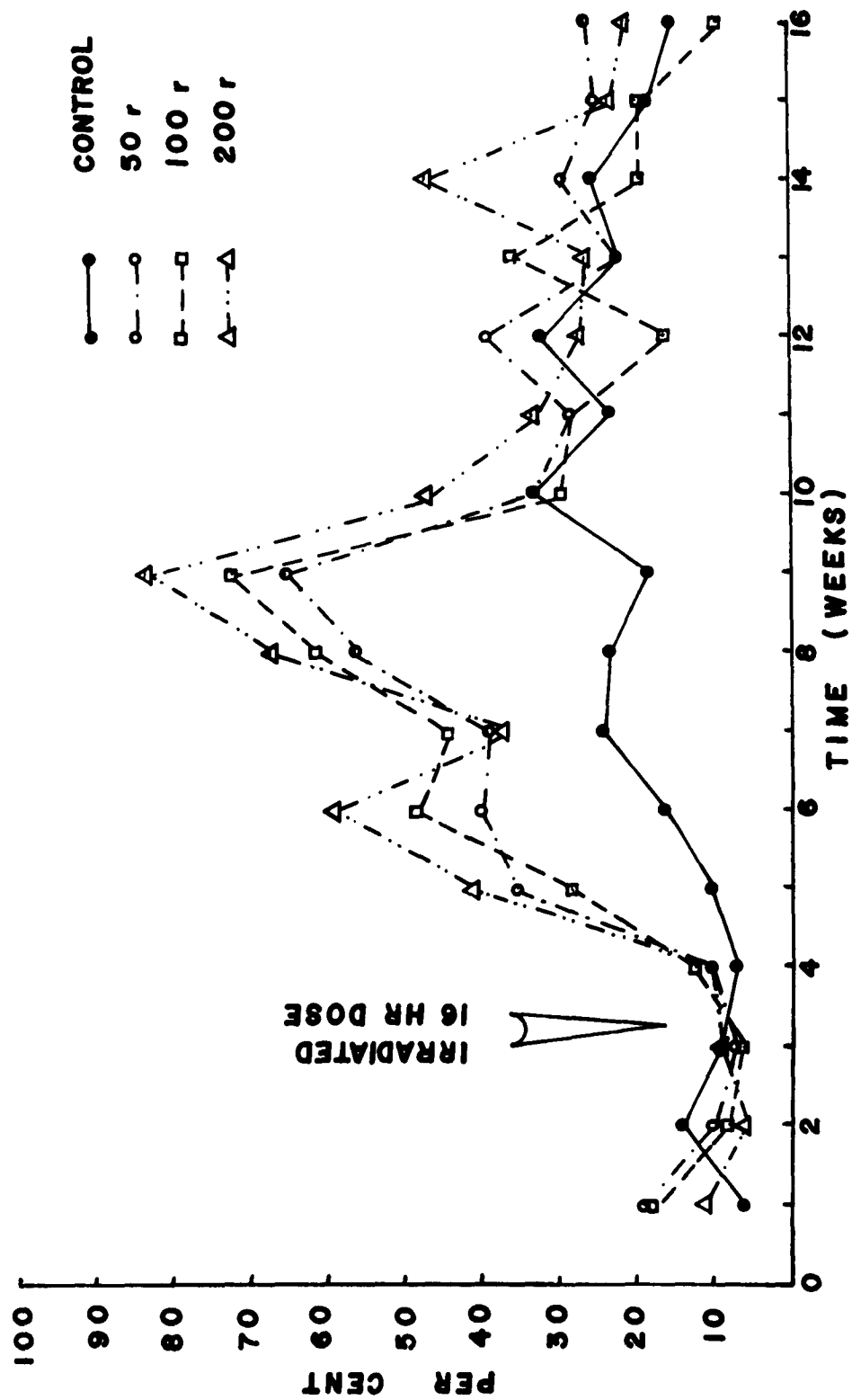
ABNORMAL SPERMATOZOA

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APPENDIX I

The following studies were carried out by graduate students under the direction of the senior investigators. While these investigations were not supported by contractual funds, they did utilize the radiation facility and some equipment supplied by the U. S. Army Research and Development Command. Since these studies have a direct bearing on the general aim of the investigation, that is, the effect of continuous low intensity radiation on the mammalian species, a presentation of these studies is included in this annual report.

EFFECTS OF CONTINUOUS LOW INTENSITY RADIATION

ON THE COTTON RAT

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Up to the present, investigations at this laboratory were carried out using laboratory animals which had been maintained for many generations in supervised colonies. It is quite possible that in these animals the response to continuous radiation would be different than in the wild species. Therefore, it was decided to trap some cotton rats (Sigmadon hispidus), native to North America, to subject their offspring to low intensity chronic radiation, and to compare the manifestations of radiation injury to those observed in albino rats. In body size, the albino rat is comparable to the cotton rat.

Methods

The first filial generation (F_1) of cotton rats trapped in the wild and bred in the laboratory was sexed and weighed at birth. The animals were weaned at 10 days of age and maintained thereafter on Purina Laboratory Chow in wire mesh cages. At the age of 40 days six male and six female cotton rats (F_1 generation) were mated and placed into the radiation chamber in each of four radiation levels, 0, 5, 10, and 20. Those 48 animals constituted the group on which this study was made. They were allowed to breed, and the young (F_2) were weighed, sexed and observed for the presence of any external anomalies. Blood samples were taken from the F_1 rats at the end of 90 days. Immediately thereafter they were killed and the tissues removed and fixed in formaldehyde. After 24 hours all tissues were weighed and the organ body weight ratios calculated. Histological sections were made of the ovaries, testes and adrenals and stained with hematoxylin and eosin. A count of the follicles and corpora lutea was made of the ovaries, and the relative abundance of the different types of cells present in the testes was determined.

Results and Observations

(1) Reproductive System - Since the reproductive system is a good indicator of radiation damage, emphasis was placed on the histologically observable changes in the gonads at different levels of continuous radiation. From Table 1 may be observed that only one litter was obtained from each of the irradiated groups as well as of the controls. Because of the size of the sample used and low reproductivity, no significant conclusion could be made on the number of litters and number of pregnancies observed. However, it was noted that at the 20 r level of daily radiation there were neither litters nor pregnancies. The weight of the ovaries was significantly decreased in those animals receiving higher dose levels of continuous radiation. The uterine weights decreased with increasing doses of radiation. From a count of the ovarian elements, in which the follicles were designated from 1 to 8 on the basis of their relative maturity, it may be observed that in the two lower levels of radiation and in the controls a large proportion of the Graafian follicles were in the advanced stages of maturity. The corpora lutea, indicating an ovulated follicle, were much more frequent in the groups receiving 0 and 5 r daily. It is recognized that evaluation of the radiation effects on the basis of ovarian damage is rather difficult, so these observations must be considered to be of a preliminary type.

The ratio of the testes weight to the body weight (X 1000) shows the characteristic relationship (Table 1) to continuous daily radiation. This index in the 20 r level group was 3.30, in the 10 r group 4.52, in the 5 r group 7.70, and 15.85 in the controls. The percentage of depleted tubules (those void of all spermatogenic elements) showed a significant increase in the 20 r group, while the controls showed the lowest percentage.

By using the designations of Clermont and LeBlone, it may be seen that the percentage of spermatids in stages 8-19 decreased in the lower levels of radiation, while the percentage of spermatids in stages 1-7 showed no significant variation. The percentage of the leptotene spermatocytes showed an increase in the lower levels of radiation and in the controls while the trend was the reverse for the pachytene spermatocytes. The percentage of spermatogonia showed a significant rise in the controls and in the lower radiation levels. The diameters of the seminiferous tubules, shown to be a fairly reliable indicator of radiation damage, were decreased in the two higher level groups. The epididymides, in a similar manner, showed a weight decrease in those males receiving from 10 to 20 r daily.

(2) Effects on Hematology - The Coulter Counter gave reliable blood counts after the threshold settings were determined for the blood cell sizes

of this animal. It was ascertained to be 10 for the red cell counts. From Table 2 it may be observed that there was a decreased in the red blood cell counts with increasing daily radiation levels in both the male and female cotton rats. A significant decrease in the red cell volume was observed in the females. The hemoglobin concentration was higher in those animals receiving the control and 5 r levels of daily radiation that in those maintained at the 10 and 20 r levels.

According to most reports in the literature the leucocytes are more radiosensitive than the erythrocytes. In this experiment the opposite was found to be the case; the erythrocytes were more radiosensitive than the leucocytes. From Table 2, it may be seen that there was no significant variation in the leucocyte count or in any of the types of the cells making up this hematological component. This observation held true when total leucocyte count (Coulter Counter technique), percentage of lymphocytes, percentage of neutrophils, absolute lumphocyte counts and the absolute neutrophil counts were considered. In another series of cotton rats, which have not been included in this report, it was found that 200 r of high intensity gamma radiation produced the typical leucopenia observed in laboratory mammals.

Biochemically no significant changes were shown in the blood glucose levels in any of the irradiated groups of animals. A peculiar observation was made on the cholesterol content of the blood of animals receiving 10 r daily radiation. It was significantly lower than that of any of the irradiated or control rats. These values were all statistically significant, and the biochemical determinations were made concomitantly so that the possibility of experimental error is ruled out.

Body and Organ Weights

The body weights of the different irradiated and control animals are shown in Table 3. From these data it may be observed that the only significant difference in weights is between the two sexes, a recognized expression of sexual dimorphism. The varying levels of irradiation showed no significant effect on the body weight. Of the organ weights (Table 4) the only significant changes were observed in the spleen, which followed the pattern observed in previous experiments by a decrease in size with increasing radiation doses.

Discussion:

The chronic radiation hematological response of the cotton rat, Sigmadon hispidus, to continuous low level gamma radiation was different than that of the nonnative laboratory animals, the albino rat, since the leucocyte count did not show the marked decrease exhibited by the albino rat. This decrease in the white blood cells of the albino rat is described in the first section of this report. The mechanisms involved in the radioresistance of these animals is certainly only a matter of conjecture since the leucocyte count showed the expected reaction to acute high intensity radiation of 200 r. The spleen showed marked decrease in size due to the continuous radiation; perhaps this is involved in the leucocyte homeostasis; however, there is no other specific evidence for this theory. On the other hand the elements which are responsible for the erythropoiesis are more radiosensitive than in the albino rats, in which the rbc is not affected by continuous radiation of the levels used in these experiments.

TABLE 1

REPRODUCTION

Criterion	Average and Standard Deviations (Roentgens/day)			
	20	10	5	0
Litters (%)	0	16	16	16
Pregnancies (%)	0	16	16	50
Ovary Weight (√B.W. × 1000)	0.07(0.04)+	0.29(0.11)	0.46(0.10)	0.58(0.14)
Uterus+Oviduct Weight (√B.W. × 1000)	1.09(0.83)	1.06(0.65)	2.66(0.88)	2.28(1.02)
Same+Embryo Weight	1.09	5.39	13.73	51.61
Ovarian Elements				
Follicle Type 8 (%)	5.8(3.2)	4.5(2.6)	9.6(4.8)	10.5(6.7)
Follicle Types 5-7(%)	23.5(11.7)	50.0(23.8)	36.8(17.3)	38.9(14.9)
Follicle Types 1-4(%)	60.4(27.4)	39.5(20.0)	31.4(12.3)	26.9(13.8)
Corpora Lutea (%)	9.9(2.9)	6.0(2.3)	22.1(10.5)	23.6(8.5)
Testes Weight (√B.W. × 1000)	3.30(0.95)	4.52(1.34)	7.70(1.08)	15.68(3.45)
Epididymis Weight (√B.W. × 1000)	0.38(0.26)	0.33(0.17)	0.85(0.27)	1.31(0.33)
Depleted Tubules (%)	2.52(2.41)	0.99(0.90)	0.92(0.72)	0.56(0.52)
Tubule Diameter(microns)	104(15)	104(54)	156(21)	178(25)
Stages 8-19 Spermatids (%)	21.72(15.52)	19.80(9.78)	17.74(6.54)	13.23(5.86)
Stages 1-7 Spermatids(%)	31.40(22.34)	32.41(8.65)	39.28(15.64)	35.92(14.72)
Pachytene Spermatocytes(%)	40.50(26.50)	29.00(9.99)	24.58(8.53)	24.23(12.36)
Leptotene Spermatocytes(%)	5.46(4.47)	17.67(7.54)	16.61(6.29)	24.14(11.85)
Spermatogonia (%)	0.92(0.87)	1.77(1.21)	1.81(1.07)	2.60(1.52)

( ) + Standard Deviation.

TABLE 2

BLOOD

Criterion	Average and Standard Deviations (Roentgens/day)			
	20	10	5	0
Red Cell Volume (%)				
Male	39.8(6.2)[+]	35.5(5.8)	38.9(3.9)	42.3(4.2)
Female	32.5(7.3)	33.8(4.9)	39.0(3.8)	44.5(3.5)
Hemoglobin(grams/100 ml blood)				
Male	18.1(6.4)	17.4(2.8)	25.5(4.5)	24.8(4.9)
Female	16.5(2.9)	16.7(4.3)	20.5(0.9)	26.7(5.1)
Erythrocyte Count (millions/cc blood)				
Male	5.42(0.97)	5.43(0.76)	6.58(0.30)	6.74(0.48)
Female	5.11(0.72)	5.13(0.38)	6.08(0.50)	6.56(0.53)
Leucocyte Count (millions/cc blood)				
Male	13.6(5.1)	9.3(3.3)	16.6(9.1)	11.7(3.7)
Female	18.2(9.9)	11.7(3.0)	13.3(7.6)	7.5(2.6)
Basophil Count (%)				
Male	0.6(0.3)	0.7(0.6)	0.4(0.1)	0.5(0.1)
Female	0.7(0.1)	1.0(0.1)	0.2(0.1)	0.0(0.0)
Basophil Count(Absolute)				
x Male	0.082	0.065	0.070	0.058
Female	0.127	0.117	0.026	0.000
Eosinophil Count (%) M	3.6(1.4)	5.3(2.3)	2.1(0.7)	3.5(1.5)
F	1.8(0.7)	4.6(2.3)	3.3(1.8)	1.8(0.8)
Absolute M	0.489	0.492	0.349	0.117
F	0.327	0.540	0.440	0.134

TABLE 2. (Continued)

Criterion		Averages and Standard Deviations (Roentgens/day)			
		20	10	5	0
Monocyte Count (%)	M	1.4(0.6)	2.2(1.3)	2.2(0.5)	2.3(1.2)
	F	1.8(0.8)	2.8(1.5)	2.3(0.7)	1.7(0.4)
Absolute	M	0.190	0.204	0.365	0.269
	F	0.327	0.328	0.306	0.126
Neutrophil Count (%)	M	17.2(6.0)	18.9(5.3)	16.4(3.5)	16.0(3.5)
	F	18.3(5.7)	17.9(4.8)	21.5(6.3)	14.8(5.8)
Absolute	M	2.339	1.757	2.725	1.873
	F	3.330	2.101	2.868	1.105
Lymphocyte Count (%)	M	77.2(5.6)	72.1(8.7)	78.9(15.3)	78.3(7.9)
	F	77.1(6.4)	72.9(7.0)	72.6(12.7)	81.6(9.2)
Absolute	M	10.499	6.705	13.113	9.168
	F	14.032	8.558	10.410	6.095
Mean Corpuscular Volume (cubic microns)					
Male		73.4	65.3	59.1	62.7
Female		63.6	65.8	64.1	59.4
Mean Corpuscular Hemoglobin Concentration(%)					
Male		45.5	49.0	65.5	58.6
Female		50.8	49.4	52.6	60.0
Glucose (mg/100 ml blood)					
Male		65.4(27.1)	64.0(17.1)	72.0(25.1)	63.3(23.1)
Female		65.1(14.2)	61.7(12.9)	71.0(13.9)	64.5(22.2)
Cholesterol (mg/100 ml blood)					
Male		139.5(39.2)	93.0(23.1)	120.8(19.6)	145.0(17.3)
Female		158.7(16.7)	85.8(16.6)	111.6(26.8)	150.8(32.0)

( )+ Standard Deviation.

TABLE 3.

ORGAN WEIGHTS

Criterion	Averages and Standard Deviations (Roentgens/day)			
	20	10	5	0
Kidneys Weight*				
Male	7.84(2.67)+	6.92(2.11)	7.38(3.00)	9.43(0.69)
Female	7.64(0.52)	7.39(1.08)	8.45(0.94)	8.45(0.85)
Lung Weight				
Male	6.59(2.30)	7.89(1.55)	8.50(1.71)	8.93(2.37)
Female	7.05(2.12)	8.14(2.03)	8.68(1.07)	9.32(2.29)
Heart Weight				
Male	3.62(0.56)	3.61(0.77)	4.08(1.01)	4.66(0.73)
Female	3.46(0.62)	3.79(0.82)	5.48(0.94)	4.81(0.58)
Brain Weight				
Male	9.89(1.46)	7.93(0.85)	8.95(0.46)	8.27(1.05)
Female	9.98(2.60)	10.12(1.35)	10.21(2.37)	10.57(0.99)
Liver Weight				
Male	39.22(7.90)	36.61(5.60)	40.89(9.72)	43.78(11.52)
Female	35.67(5.40)	32.23(4.79)	39.17(6.81)	37.00(9.32)
Adrenal Weight				
Male	0.30(0.01)	0.28(0.40)	0.30(0.08)	0.39(0.06)
Female	0.54(0.05)	0.61(0.08)	0.58(0.07)	0.76(0.10)
Spleen Weight				
Male	0.73(0.22)	0.66(0.11)	1.03(0.13)	0.92(0.02)
Female	0.67(0.03)	0.73(0.09)	1.03(0.13)	1.05(0.20)

( )+ Standard Deviation.

*(Note: All organ weights divided by respective body weights x 1000).

TABLE 4

BODY WEIGHTS

Criterion	Averages and Standard Deviations (Roentgens/day)			
	20	10	5	0
Birth Weight (grams)				
Male	6.3(0.5)+	6.1(0.4)	6.9(0.4)	6.5(0.4)
Female	6.6(0.1)	6.3(0.3)	6.9(0.6)	6.6(0.2)
Weaning Weight (grams)				
Male	17.4(2.6)	13.2(2.3)	16.2(3.1)	16.5(1.8)
Female	17.2(2.0)	12.9(1.7)	15.8(2.5)	16.3(1.5)
Breeding Weights (grams)				
Male	59.9(3.2)	55.2(5.0)	60.2(6.2)	69.1(7.7)
Female	53.5(3.7)	54.5(4.2)	64.3(2.9)	63.6(6.8)
Sacrifice Weight (grams)				
Male	101.8(20.8)	121.0(9.3)	115.0(11.0)	124.0(24.1)
Female	78.6(5.1)	98.2(8.8)	95.5(7.4)	98.2(15.2)

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AN INVESTIGATION OF AUDIOGENIC SEIZURE PRONE RATS AS

INDICATORS OF LOW INTENSITY CHRONIC GAMMA

IRRADIATION EFFECTS

Julian P. Cooke

Introduction

In 1924 Donaldson described an "almost maniacal running and jumping" reaction in a certain number of rats when the animals were subjected to the jingling noise of keys. Since that time, many types of sound have been used to induce patterns of responses that deviate from expected typical scare reactions. The dramatic convulsive reactions displayed by certain animals from a population to such a stimulus have resulted in the descriptive phase "audiogenic seizure," a term introduced by Morgan and Waldman (1939) and now the most widely accepted name for this condition (Finger, 1947).

Audiogenic seizure may be manifested as an undirected running, a tonic, or a clonic reaction. The seizure may be interrupted by a quiet period or may be followed by a stage that resembles coma, or it may continue at a reduced rate until the animal is exhausted. The most characteristic reaction is the convulsion; this reaction may result in a tonic extension of the appendages which is reminiscent of decerebrate rigidity, of clonic beating of the limbs, or a combination of these two actions. The animal may lapse into a state of unconsciousness, or it may die immediately. Nonseizure prone animals appear to be only slightly irritated by the high noise levels.

Many factors play a significant role in the frequency and degree of audiogenic seizure, such as age (Frings, Frings, and Kivert, 1951; Vicari, 1948; Dice and Barto, 1952; Ginsburg and Hovda, 1947), sex (Miller, Ginsburg, and Potas, 1952; Ginsburg and Roberts, 1951), strain or genetic background (Witt and Hall, 1949; Fuller and Williams, 1951), nutrition, temperature, intensity and frequency of the stimuli. The seizure has been shown to be one that is not learned and one to which the animal cannot become adapted. The reaction has also been shown to be different from a simple fright reaction.

Some physiological and biochemical differences have been reported to exist between susceptible and nonsusceptible strains of rats. Hofeld (1948)

reported that a rise in the BMR takes place during the audiogenic seizure. He notes, also, that the seizure is followed by a comatose state, during which time the animal's BMR is subnormal.

In the past, hematologic changes such as a reduction in the total number of lymphocytes found in the peripheral blood have been found to be the most sensitive indicators of acute or subacute damage following radiation originating from external sources (Jacobson, 1954). But this technique is limited in that the total amount of radiation must be extremely high.

Investigation of the effects of radiation upon the nervous system per se has shown it to be relatively radioresistant, with large acute doses of 1000 r or more necessary to cause observable neural degeneration in many different mammals (Arnold, Bailey, and Laughlin, 1954; Clement and Holst, 1954; Hicks and Montgomery, 1952; McLaurin et al., 1955). Histological examination of the neural tissue seems to offer no solutions to the problems arising from low intensity chronic gamma irradiation unless the cumulative dose is high. Using rats that were aged eight hours to 15 days, Yamazaki et al. (1960) showed that when a radiation level of 125 r was given to the head, more neurological signs were evident when irradiated during one of the first four days than when irradiated on the fifth day, thus showing that the young nervous system is more radiosensitive than that of the maturing young system.

Lebedinsky (1956) has reviewed Russian literature which indicates that X-ray exposure to doses as low as 0.1 r causes functional alterations of the central nervous system. Caster, Redgate, and Armstrong (1958) have suggested that if these findings could be confirmed, it would seem that the central nervous system might contain the most radiosensitive tissues of the body.

Equipment for Tests

Test Chamber — The test chamber consisted of a No. 2 galvanized washtub, approximately 60 cm in diameter and 27 cm high, which is mounted in a plywood box. The tub rests on a 3/4-inch thick piece of foam rubber; and for purposes of insulating and of eliminating sound reverberations, the space between the tub and box is packed with small wood shavings. A hinged cover was made of 0.5 cm thick plexiglas.

Illumination within the chamber was provided by two 7.5-watt lamps mounted on the underside at the back edge of the top. Ventilation was provided by two

one-half-inch pipes extending through the wood portion of the top. The
metal tub was first painted with a rust inhibiting metal primer and then
finished with a nonglossy black metal paint.

Sound Stimulus — The equipment that was used to produce stimuli for
audiogenic seizure testing consisted of a recording microphone, audio-
generator and motor, tape recorder and tape, amplifier, crossover, speaker,
voltmeter, and sound level meter (Figures 1 and 2).

Standardization Procedure — To establish standards for testing, a three-
step procedure was followed. First, the variable audiogenerator was set
at frequencies of 5,000 to 11,000 cps, in steps of 1,000. Each frequency
signal was recorded on tape. Each tape signal was then amplified and the
signal played into the closed sound chamber that housed a sound level
meter. The voltage, as measured across the speaker terminals, was ad-
justed to the following series of values by means of the volume control on
the amplifier: 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and
9.0 volts. At each voltage the sound level within the test chamber was
measured from three different locations, and a mean sound level in decibels
was calculated.

The second step consisted of the testing of 122 Wistar-strain adult rats,
117 to 120 days old at the beginning of the experiment, for audiogenic
sensitivity by exposing each animal to an oscillating set of frequencies
from 8,000 to 11,000 cps, with an oscillation of 120 cycles/min. The
noise level was 122 db ± 0.1 db (over-all mean). The rats were tested
for audiogenic seizure sensitivity at two-day intervals for a total of four
trials. Individual records were maintained for each animal.

The same 122 rats were then tested to auditory stress with oscillating
frequencies (120 cycles/min) between 5,000 and 8,000 cps at two-day
intervals for a total of four trials.

The third step consisted of testing the animals at frequencies of 8,000
to 11,000 cps superimposed upon frequencies of 5,000 to 8,000 cps. This
was made possible by the use of a stereo recording of each set of frequencies,
and the combined frequencies were fed into the one high-frequency speaker.
Each series of tones was played at the same volume (as indicated by the
voltage), and the over-all voltage was raised to five volts at the speaker
terminals, a level equal to 122 db ± 0.5 db.

Procedure

One hundred and twenty adult Wistar-strain rats which had been tested
12 times for audiogenic seizure (used in standardization study; two of the
original 122 animals were removed for breeding purposes) served as the
colony from which animals were selected for this study. Rats which had
given no previous indication for seizure tendency were divided into six
groups. Group I, Marked C, was used as a control. The other five groups
were irradiated with graded concentrations of gamma radiation at the rate
of 20 r/day and were then tested for audiogenic seizure. The following
groups were established and given the irradiation indicated:

> Group I (animals C) - Control - no irradiation.
> Group II (animals P) - Total irradiation = 20 r.
> Group III (animals R) - Total irradiation = 40 r.
> Group IV (animals S) - Total irradiation = 80 r.
> Group V (animals T) - Total irradiation = 160 r.
> Group VI (animals V & W) - Total irradiation = 320 r.

Following irradiation, all rats were stressed with a noise source con-
sisting of oscillating sine wave frequencies of 5,000 to 8,000 cps superim-
posed upon frequences of 8,000 to 11,000 cps at a noise level of 122 db.

A seventh group was established composed of 22 adult Wistar-strain rats
which had a history of running, seizure, or a combination of these responses
to four trials of audiogenic seizure testing. This group was irradiated with
a total of 25 r, administered at the rate of 5 r/day for a total of five days.
The rats, marked Y and Z, were then tested with the same auditory stimuli
for four trials.

Results

A summary is presented in Table 1 of the responses of nonseizure prone
adult Wistar-strain rats to audiogenic seizure testing following the admin-
istration of chronic gamma irradiation totaling either 20, 40, 80, 160, or
320 r (administered at the rate of 20 r/day). These data show that 18.5
percent of the male and 11.1 percent of the female animals react in some
way to the sound stimuli following 20 r or more. The same percentage
(7.4 percent) of the irradiated males and females entered the complete
seizure with tetany. It is important to consider that animals which enter
the complete seizure with tetany are not considered with the "run-only"
group.

TABLE 1

Summary of Responses of Nonseizure Prone Wistar-Strain Rats to Audiogenic Seizure Testing* Following Administration of Chronic Gamma Irradiation** to Adult Animals

| Sex | Total Radiation | | | | | | Total | % | Signif. x^2 Test |
	0 r	20 r	40 r	80 r	160 r	320 r			
I. Reaction: Run Only + Tetany/Total Number of Animals.									
♂	0/5	0/5	1/5	1/5	2/5	1/7	5/27	18.5	P<0.01
♀	0/5	1/5	0/5	0/5	0/4	2/8	3/27	11.1	P<0.01
II. Reaction: Tetany Only/Total Number of Animals.									
♂	0/5	0/5	0/5	1/5	1/5	0/7	2/27	7.4	P<0.01
♀	0/5	1/5	0/5	0/5	0/4	1/8	2/27	7.4	P<0.01
III. Reaction: Run Only + Tetany/Total Number of Trials.									
♂	0/20	0/20	3/20	3/20	6/20	1/21	12/101	11.8	P<0.01
♀	0/20	3/20	0/20	0/20	0/16	5/24	8/100	8.0	P<0.01
IV. Reaction: Tetany Only/Total Number of Trials.									
♂	0/20	0/20	0/20	3/20	2/20	0/21	5/101	5.0	P<0.01
♀	0/20	3/20	0/20	0/20	0/16	2/24	5/100	5.0	P<0.01

*Auditory stress was provided by tones of 5,000-8,000 cps (oscillating at 120 times/min) superimposed upon frequencies of 8,000-11,000 cps (oscillating at 120 times/min). Mean noise level was 122 db.

**Irradiation dose was delivered at the rate of 20 r/day (23 hours per 24-hour day).

If the total number of trials is used as the basis for consideration, then 11.8 percent of the males and 8.0 percent of the females display either of these reactions following irradiation. In the case of the tetany reactions, 5 percent of either sex entered the complete reaction with tetany.

None of the controls, tested the same day as the 320 r group, displayed any type of reaction. When the x^2 test was employed, the level of confidence in all cases was significant below the one percent value. The data were obtained by testing with the complex stimuli.

When Table 2 is considered, it can be seen that no sifnificant change (P>0.05) took place in the latency period for any type of reaction in adult irradiated rats that had, previous to irradiation, displayed some form of seizure. In this group, a total of 25 r was administered over a total period of five days. The latency for a reaction prior to and following irradiation was 23.5 seconds and 22.0 seconds, respectively. The latency periods for a tetany reaction before and after irradiation were 52.6 and 49.5 seconds, respectively. Using the t-test, the reduction in time was not significant (t = 0.72; P>0.05).

The number of times an animal entered tetany out of the total number of trials was only slightly reduced following irradiation, a mean value from 2.2 to 1.9, a value which was not significantly changed when the χ^2 test is employed to evaluate the level of confidence (P>0.05). Neither was there any significance to the total number of times that an animal entered either of the types of reaction; this value was reduced from 3.6 times prior to irradiation to 3.3 times following irradiation (total of four trials).

Following either the run-only reaction or the full seizure, a period of time exists during which the rat appears quite rigid as if in a decerebrate rigidity. In this study this condition is spoken of as "tetany." The animal may lie or stand in a staunch manner with the tail extended and quite rigid. If the rat's tail is raised by the experimenter, the tail tends to remain in this position. Figure 3 shows this condition. A second type of response is evident in many animals following either the run-only or the full reaction, i.e., the animal can be "moulded" or placed into usual positions. This plastic-like condition has been described by Maier (1939) and is shown in Figure 4.

In the past, most workers have not considered the running reaction as a type of seizure. It would appear, however, that the running reaction should be classified as a type of seizure since both the running reaction and tetany are generally followed by some degree of rigidity and plasticity.

TABLE 2

Summary of Responses of Adult Seizure Prone Wistar-Strain Rats*
(6.5 months old) to Audiogenic Seizure Testing Following
Administration of 25 r Gamma Irradiation**

Marking: Rats Y and Z
Number: 22

	Response Time (seconds). Before Irrad.	Response Time (seconds) After Irrad.	Test of Confidence	
Latency period for any type of reaction	23.5	22.0	$t = 1.1$	$P>0.05$
Latency period for tetany reaction	52.6	49.5	$t = 0.72$	$P>0.05$
	Before Irrad.	After Irrad.		
Number of times animal enters any type reaction	3.6	3.3	$x^2 = 0.48$	$P>0.05$
Number of times/4 animal enters tetany only	2.2	1.9	$x^2 = 0.9$	$P>0.05$

*All rats had previously displayed sensitivity one or more times to audio-genic seizure testing by either running, tetany, or a combination of these responses.

**Administered at the rate of 5 r/day for five days (23 hours per 24-hour day).

Discussion and Conclusions

Whereas the causes and physiological changes associated with audiogenic seizure are as yet not completely clarified, the similarity of this type of seizure to epilepsy of the human suggests an involvement of a neurological-type disorder. If audiogenic seizure, then, is classified as a type of neurological disorder, any factors which affect the syndromes of seizure will, either directly or indirectly, modify the neurological apparatus to some extent. Most workers have insisted that the nervous system is one of the most resistant to radiation damage since total dosages of several hundred roentgens are necessary in order to demonstrate any histological damage to this system. This study has served to help strengthen the belief that the nervous system is not as radioresistant to gamma irradiation as previously believed and that damage to the nervous system, caused by very low levels of chronic irradiation, can be demonstrated as a change in the audiogenic seizure picture.

In the case of adult nonseizure prone Wistar-strain rats which were administered a low level of chronic gamma irradiation, some important changes in the frequency of audiogenic seizure were noted. However, adult rats which had shown some degree of seizure before irradiation displayed no change in seizure pattern afterward.

It thus appears that the chronic irradiation of the adult Wistar-strain rat does cause an increase in the frequency of the run-only reactions as well as in the frequency of complete seizure. The adult animal, however, does not appear to be as reliable an indicator for low level irradiation damage as would the prenatally irradiated rat or the mouse. The fact that the chronic administration of 320 r (delivered at a chronic rate of 20 r/day) caused no greater incidences of either type of audiogenic seizure reactions than did lower levels would tend to point to an all-or-none involvement of the nervous system. It suggests, also, that the total amount of chronic gamma irradiation necessary to initiate a change in the seizure pattern lies below a cumulative dose of 20 r.

Thus, the hypothesis that was proposed for this study has been fulfilled. With the development of a standarized technique which has been completed with this study, either rats or mice may be used as indicators of low level radiation damage if changes in the response to audiogenic seizure testing are considered.

Figure 1. Overhead view of sound stimulus equipment for audio-
genic seizure testing. Sitting atop the amplifier is the
voltmeter.
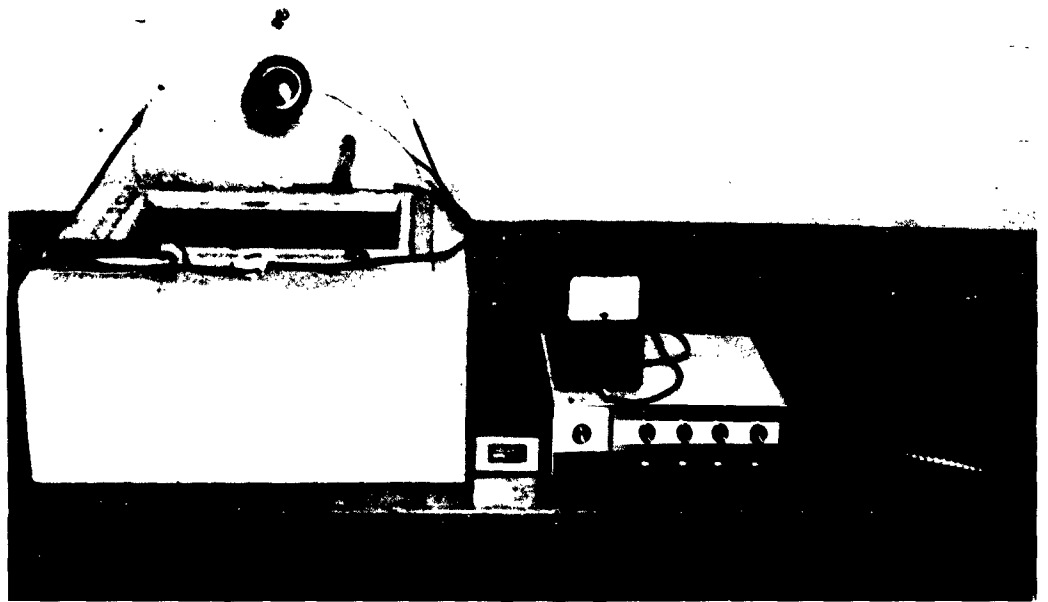
Figure 2. A schematic view of the testing equipment. The tape
mechanism is shown in place for recording the pure
sine wave frequencies.

speaker

Test Chamber

Cross-over
Filter

Amplifier

Tape   Mechanism

Variable Audiogenerator   Motor
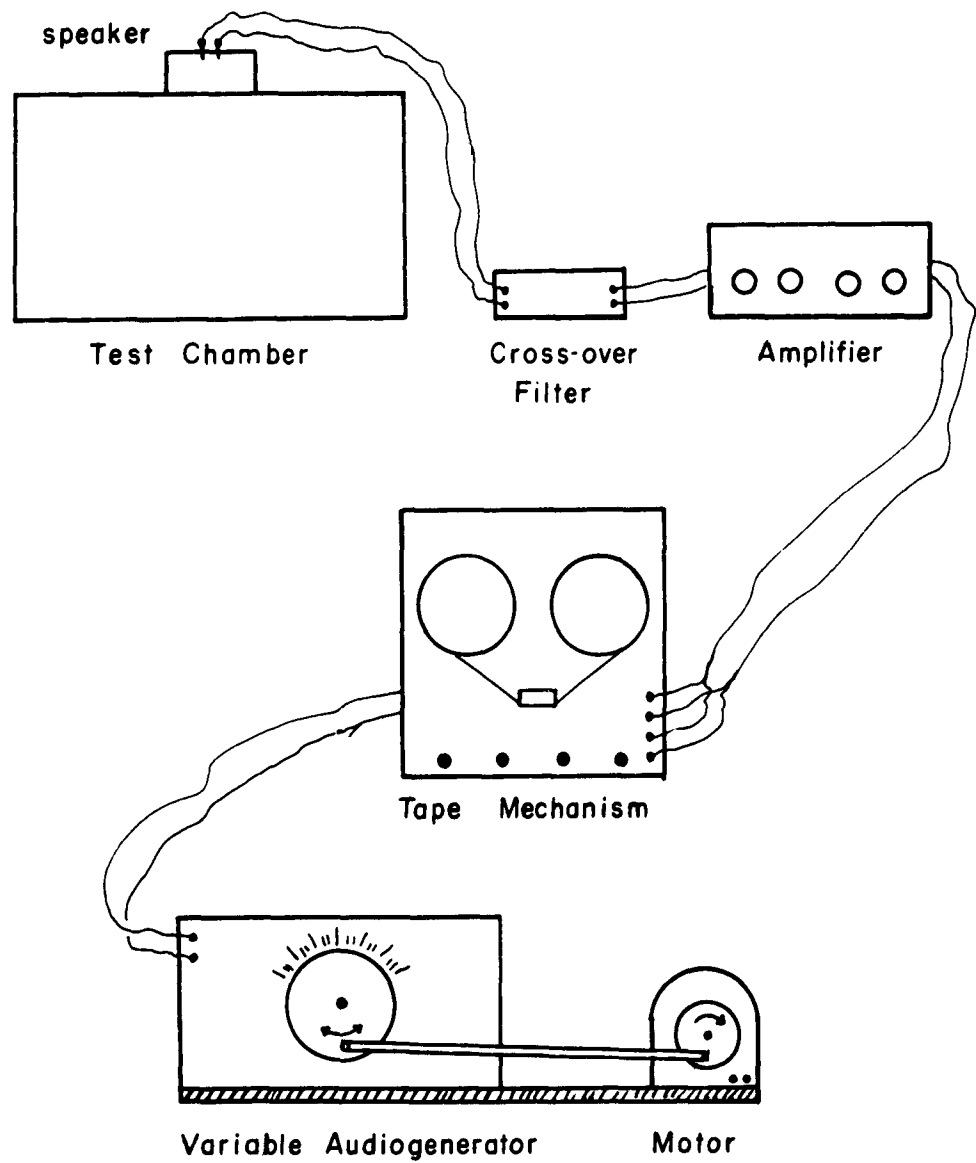
Figure 3. Two different Wistar-strain rats are shown in full seizure
(tetany) following auditory stimulation. Note the rigid
position of the tail and the taut extension or retraction
of the legs.

Figure 4. A Wistar-strain rat is shown in a plastic-like stage
which may follow either the full audiogenic seizure
(tetany) or the audiogenic run-only reaction. The rat
may remain in any position in which it is placed by
the experimenter.

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INVESTIGATION ON THE PROTECTIVE ACTION OF B-AMINO-

ETHYLTHIOSULFURIC ACID ON RATS GIVEN REPEATED

MACROFRACTIONATED DOSES OF

GAMMA IRRADIATION. II.

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Introduction

The purpose of this investigation was to study the protective action of B-aminothiosulfuric acid on the hematology, weight gain, and organ body weight ratios in rats subjected to repeated macrofractionated doses of total-body irradiation. Radiation was administered at the dose rate of 50 r per 20-hour day.

The effectiveness of B-aminoethylthiosulfuric acid (WR-361) as a protective agent against acute irradiation is established by extensive tests on mice. Holmberg and Sörbo (1959) tested the protective effect of B-aminoethylthiosulfuric acid on mice irradiated with a lethal dose of X-rays and found that the drug (determined as LD-50 by intraperitoneal injection into albino mice) was found to be $0.90 \pm .02$ g/Kg body weight. There are few investigations reported in the literature, however, for the effectiveness of WR-361 as a protective agent against chronic continuous irradiation. As reported in a previous communication (Brown, et. al, 1962) no protection was afforded by WR-361 against loss of testes weight, leucopenia, or lymphocytopenia by albino rats exposed to 20 r per day of gamma irradiation.

Some preliminary tests on mice have indicated that chronic toxicity may outweigh the protective action (Jacobus, 1961). In order to establish the maximum level tolerated by the albino rat when fed this substance, a series of tests was undertaken. Reported in a previous communication, (Pace, et. al, 1962), these tests indicated that 50 mg. of WR-361 given daily with the food was the maximum amount that could be tolerated without undue decrease in body weight.

Albino rats of the Holtzman strain weighing approximately 280 ± 10 g. were utilized for the test and were divided into eight groups of eight animals each. The following shows the treatment for each group:

	WR-361	Irradiation
Group I	0	50 r/day
Group II	50 mg/day	50 r/day
Group III	75 mg/day	50 r/day
Group IV	100 mg/day	50 r/day
Group V	0	0
Group VI	50 mg/day	0
Group VII	75 mg/day	0
Group VIII	100 mg/day	0

For those groups to which the drug was administered the substance was weighed out and mixed with 15 g of finely ground Purina Laboratory Chow immediately before feeding. Each animal was offered 15 g of food daily. Although this is below the maximum daily requirement, it was thought to be adequate to maintain body weight in this strain of rats on the basis of previous experiments. By employing Fragley feeders, the amount of food lost was negligible. The submaximal amount was used to assure complete consumption of the food and the drug. As shown in Tables 1 and 2, this was not the case, those animals on higher drug level and exposed to irradiation, consumed less than 15 grams of food and therefore, less drug. The food container was weighed each day, and the amount of residual food recorded. All animals were weighed weekly.

At the end of five weeks the irradiated animals given the higher drug levels began to show a decline resulting in several deaths. It was decided to remove all animals from the drug and irradiation and to follow the hematological and weight recovery. It was thought that this might reveal a possible protection to the hemopoietic system by the drug. Data were obtained during the following three weeks, or until the weight and hematological values of the controls began to approach a normal level. During this 3-week period all animals were given food and water ad libitum.

Hematological Procedures

Hematological procedures were carried out at the time of initiation of the experiment and each week thereafter. In this procedure, the animals

were anesthetized lightly with ether, and a small segment of the tail removed with a razor blade. Approximately 1 ml. of blood was caught in a small test tube, and EDTAP was added to prevent coagulation. The wound was then cauterized and the animal returned to its cage. The red and white cell counts were determined by use of the Coulter Counter. The hemaglobin was determined by the cyanmethemoglobin technique employing the B and L Spectronic 20. The hematocrit was determined by the method of Guest-Silver.

Terminal Procedure

At the termination of the experiment, the animals were given an anesthetic injection of Nembutal and the organs were removed, weighed, and fixed for histopathological studies. These included the testes, liver, spleen, kidney, adrenal, and heart. Smears of bone marrow were made, and fresh imprints of spleen and lymph node were prepared.

Results and Observations

1. Body Weight: (Figure 1)

Since weight loss was observed in the group receiving no drug or irradiation during the initial five weeks it might be deduced that 15 g. of food was not sufficient to maintain the weight of animals of this size (280 ± 10 g.). In fact, at the end of five weeks, the average weight of all groups, both irradiated and nonirradiated was approximately the same. However, weight loss was more rapid in those groups receiving both irradiation and drug.

At the end of the initial five weeks when irradiation and drug was withdrawn and an ad libitum diet was provided both irradiated and nonirradiated controls recovered their initial weight in three weeks. All animals receiving drugs, both irradiated and nonirradiated did not return as rapidly to their initial weights. This would indicate an incipient damage to the animals by the drug itself. Hematology; (Figures 2-6).

As seen in Figure 2, 50 r per day equally depressed all irradiated groups well below the groups receiving no irradiation. No explanation can be given for the drop in leucocyte count for the control group receiving no drug or irradiation. However, the leucocyte count of the nonirradiated groups receiving the two highest levels of drug (75 and 100 mg per day) were well above the nonirradiated group receiving no drug or the lowest level of drug (50 mg per day).

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This would indicate an inflammatory condition by the two higher drug levels. The leucocyte count for these two groups remained high even following three weeks of drug withdrawal. This was not observed in the irradiated groups possibly because of the failure of the radiation damaged hemopoietic system to respond. No evidence of radiation protection by the drug was observed on the leucocyte count during radiation exposure or during the recovery phase.

Lymphocyte and neutrophil values are shown in Figure 3. In the irradiated groups, the lymphocyte-neutrophil ratios are shown to be depressed to a greater extent in those animals receiving drug over that of the group receiving no drug. Recovery of a normal balance in lymphocytes and neutrophils following withdrawal of irradiation and drug is also slower in those groups receiving the drug. No differences were observed in the lymphocyte-neutrophil ratios of the nonirradiated groups, both treated and untreated with drug. No protection is indicated by WR-361 on depression of lymphocyte count in the irradiated groups.

50 r per day of gamma irradiation considerably depressed the erythrocyte count during the five weeks of exposure (Figure 4). The depression was greatest in those irradiated groups receiving drug treatment. A recovery to normal erythrocyte values was observed in the irradiated group receiving no drug after 3 weeks of radiation withdrawal. This was not the case of the irradiated drug treated groups. This drug effect on erythrocyte depression was also observed in the nonirradiated groups. Hemaglobin and hematocrit values (Figures 5 and 6) varied consistently with erythrocyte count.

Mortality and Pathology:

Figure 7 shows the percent mortality during the experiment. Few mortalities were observed in any groups except those two irradiated groups receiving 75 and 100 mg. of WR-361 per day. One may assume that the combined effects of these drug levels and irradiation were more detrimental than the effects of the drug or irradiation alone. The signs observed just before the animals became moribund were diarrhea and a bloody nasal discharge.

The following is a pathology report on animals from another experiment (RBL-33) receiving 50 mg. of WR-361 per day.

Histopathological Observation: Walter F. Mestanza, DVM.

The Group I, receiving both the drug (50 mg) and 20 r daily radiation, presents the following microscopic findings.

Testes and Epididymides –

Marked atrophy of the seminiferous tubules. Total degeneration of the germinal cells for the majority of cases there is absence of spermatozoa in the seminiferous tubules and epididymides.

When spermatozoa are present they are found in the cauda epididymis in a dense, mucinous, eosinophylic material, and in some cases mixed with cellular detritus.

Edema in the intertubular tissue.

Relative increase of Leydig cells.

Presence of cellular debris in the epididymides.

In one case, the small arteriole contained polynuclear neutrophiles and in another there is a mild perivascular lymphocytic infiltration in the tunica albuginea.

One rat presented necrosis of a few tubules in the proximal pole of the testicle with presence of some macrophages containing hemosiderin.

Spleen –

Mild congestion and depletion of lymphoid tissue.

Scanty nuclear pycnosis and clumping of lymphocytes.

Kidney –

Slight pycnosis in some glomeruli.

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Mild congestion.

Fatty infiltration in some convolute tubules in several animals and presence of hyaline material in others.

Presence of hyaline casts either in the convolutes of collecting tubules.

The liver, heart, thyroids and parathyroids showed normal appearance in all the animals of this group.

The Group II, receiving 20 r daily irradiation and no drug, show more or less similar lesions than those presented in Group I.

Testes and Epididymides –

Atrophy of the seminiferous tubules.

Degeneration of the germinal cells.

Absence of spermatozoa in testes and epididymides in the majority of animals in this group. When spermatozoa are present, they are in the cauda epididymis and almost always accompanied by cellular detritus.

Relative increase of Leydig cells.

Mild intertubular edema.

In one animal, the small arteriole contained abundant polynuclear neutrophils and in other necrosis of some seminiferous tubules were found.

Spleen –

Mild congestion and depletion of lymphoid tissue.

Scanty pycnosis and clumping of lymphocytes.

Kidney –

The majority of animals present no microscopic abnormalities. Those showing lesions present:

Mild tubular degeneration.

Shrinkage of some glomeruli with scanty pycnosis.

Scarce hyaline substance in some descending tubules.

The heart, liver, thyroids and parathyroids show no visible abnormalities.

The Group III, receiving only 20 r per day and no drug, shows no alterations in testes, spleen, heart, kidneys, thyroids and parathyroids, with exception of one animal who presented small hemorrhages in the renal pelvis and abundant mineral deposits.

The control group (IV) presents no significant lesions in the tissues examined.

Average Organ Weights

Testis weights were equally depressed in all irradiated groups, both treated and untreated with WR-361. The testes weights were greater in the unirradiated groups, no difference being noted in those treated or untreated with WR-361. The weights of the liver, heart, adrenal, or kidney did not vary appreciably between groups. The spleen weight was less in those drug treated irradiated groups. However, just the reverse was observed in the nonirradiated groups; those groups receiving drug had an increased spleen weight proportional to amount of drug administered.

Summary

1. Male albino rats maintained on 15 grams of food per day were exposed to 50 r per day of gamma irradiation. No protection was afforded by 3 levels of B-aminoethylthiosulfuric acid to radiation induced body weight loss, leukopenia, lymphocytopenia, erythropenia or testes weight loss, at the levels of 50, 75, and 100 mg per day.

2. WR-361 enhances the body weight loss and erythropenia of animals receiving 50 r of gamma irradiation per day.

3. At the end of 5 weeks exposure to 50 r per day of gamma irradiation and treatment with WR-361, all groups were placed in control conditions on ad libitum food. Recovery of initial body weight was not as rapid in those irradiated and nonirradiated groups given 75 and 100 mg of drug per day.

4. Leucocytosis was observed in the nonirradiated groups receiving 75 and 100 mg of WR-361 per day. This condition persisted during the three-week period of drug withdrawal. This was not observed in the irradiated groups.

5. WR-361 increased the effects of radiation in depressing the lymphocyte-neutrophil ratio.

6. Mortality and morbidity was greatly increased in those animals receiving 75 and 100 mg. of WR-361 per day.

TABLE 1

AVERAGE WEEKLY FOOD CONSUMPTION (PER RAT)

Group	Week								
	1	2	3	4	5[2]	6	7	8	9
I 50 r/day; No Drug	93.0	102.7	103.7	104.6	104.1	105.0	105.0	105.0	105.0
II. 50 r/day; 50 mg/Day	80.0	110.4	103.0	103.7	104.1	105.0	105.0	105.0	105.0
III 50 r/day; 75 mg/Day	78.5	97.7	103.0	99.6	96.4	105.0	105.0	105.0	105.0
IV 50 r/day; 100 mg/Day	71.9	88.5	94.7	93.6	95.1	105.0	105.0	105.0	105.0
V No r; No Drug	102.3	105.0	105.0	105.0	105.0	105.0	105.0	105.0	105.0
VI No r; 50 mg/Day	102.5	104.6	105.0	105.0	105.0	105.0	105.0	105.0	105.0
VII No r; 75 mg/Day	93.4	105.0	105.0	105.0	105.0	105.0	105.0	105.0	105.0
VIII No r; 100 mg/Day	89.4	102.8	102.8	104.5	103.2	105.0	105.0	105.0	105.0

[1] The amount of drug indicated represents that amount of WR-361 made available to each rat. The average amount of drug actually consumed is shown in Table 2.
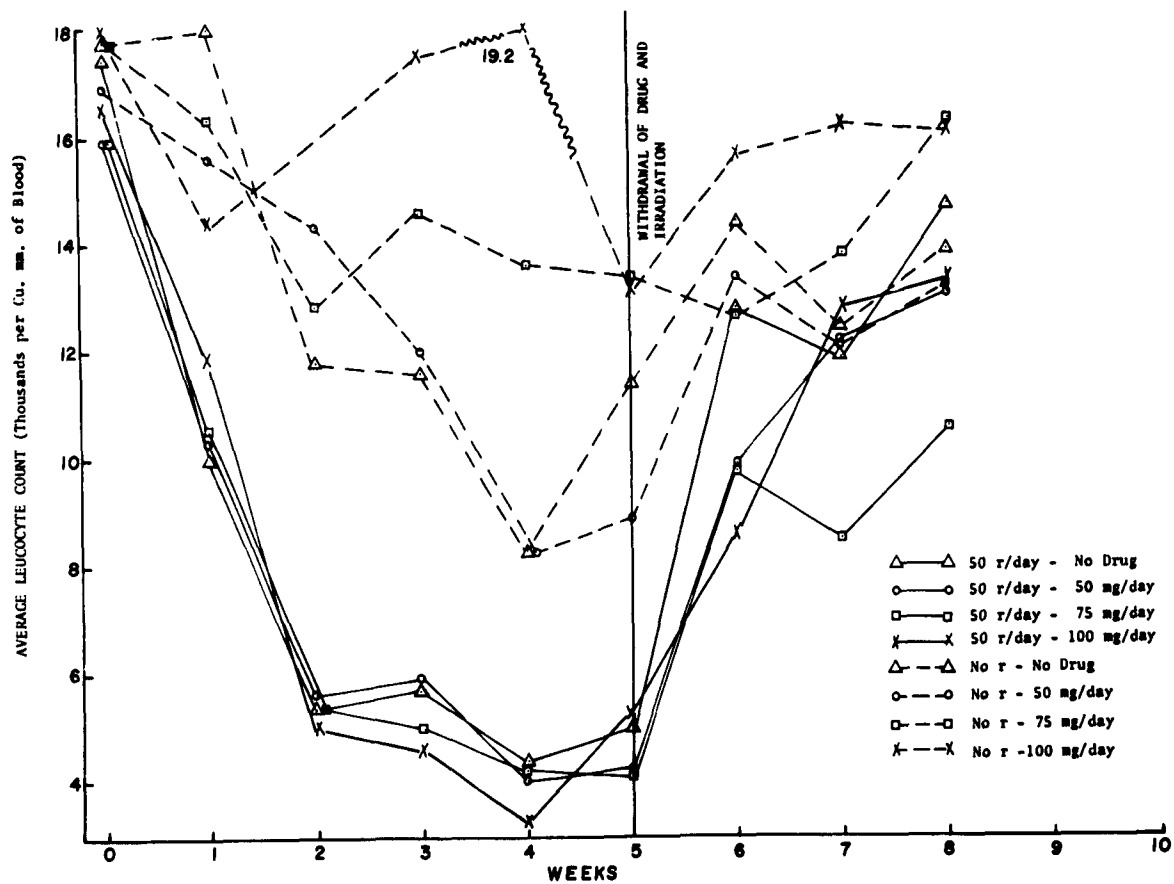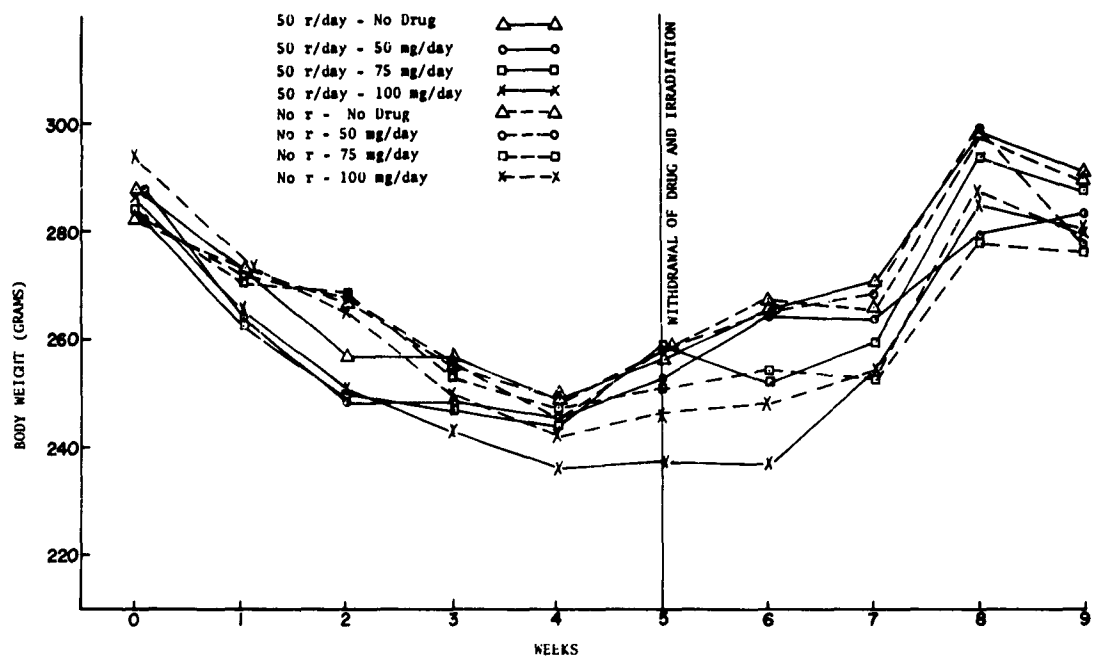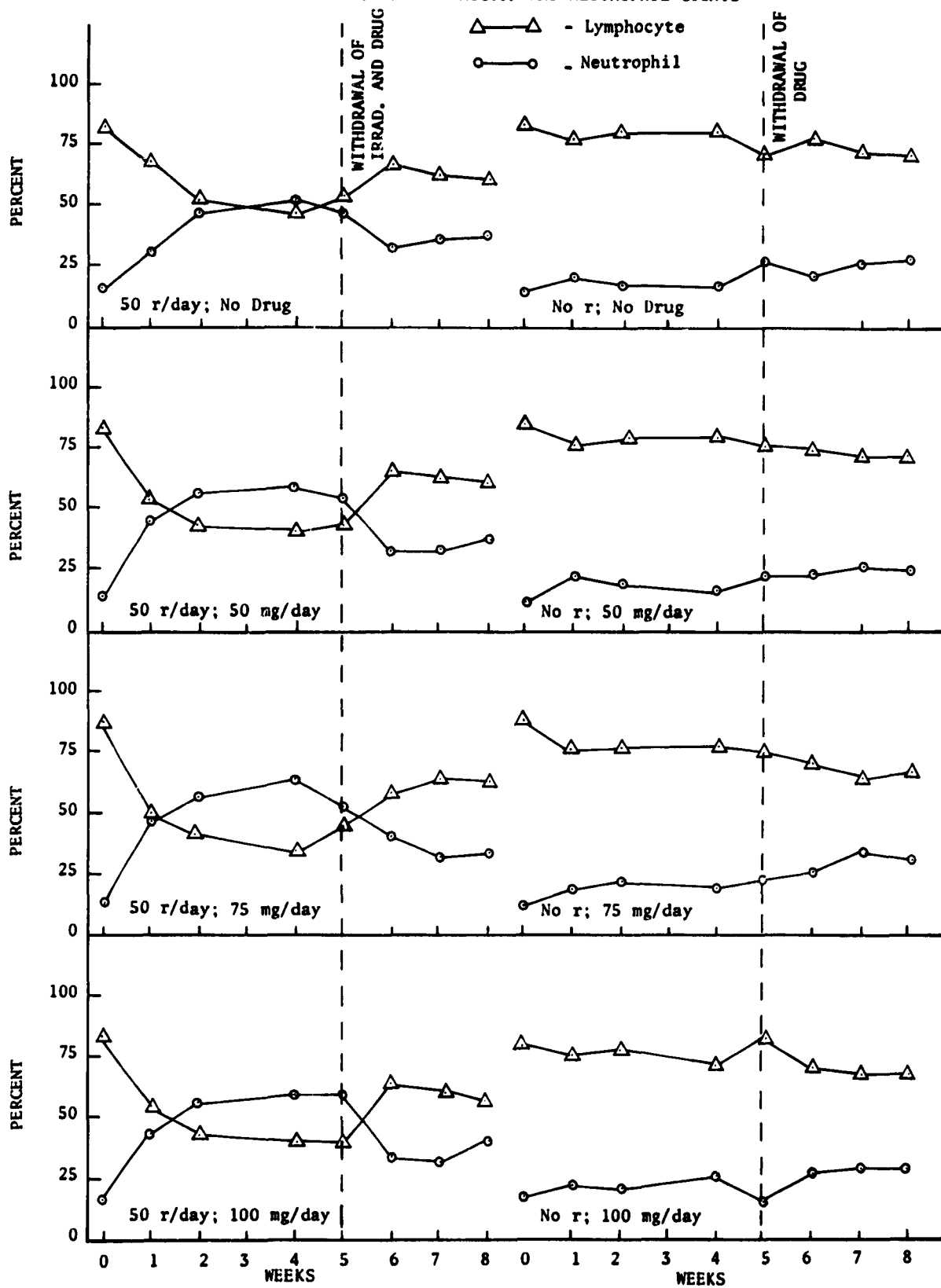
[2] At the fifth week, the irradiation exposure and drug was withdrawn.

TABLE 2

AVERAGE DAILY CONSUMPTION OF WR-361 (PER RAT)

Group	Week									Average
	1	2	3	4	5	6	7	8	9	
I 50 r/day; No Drug II	-	-	-	-	-	-	-	-	-	-
50 r/day; 50 mg/Day[1] III	38.1 mg	44.6 mg.	49.0 mg.	49.3 mg.	49.5 mg.	-	-	-	-	46.1 mg.
50 r/day; 75 mg/Day IV	56.0	69.8	69.4	71.1	68.8	-	-	-	-	67.0
50 r/day 100 mg/Day V	68.4	81.4	90.2	89.1	90.6	-	-	-	-	83.9
No r; No Drug VI	-	-	-	-	-	-	-	-	-	-
No r; 50 mg/Day VII	48.9	49.7	50.0	50.0	50.0	-	-	-	-	49.7
No r; 75 mg/Day VIII	66.8	75.0	75.0	75.0	75.0	-	-	-	-	73.4
No r; 100 mg/Day	85.1	97.9	97.9	99.5	98.3	-	-	-	-	95.7

[1] The amount of drug indicated in this column represents that amount of WR-361 made available to each rat.

TABLE 3

AVERAGE ORGAN WEIGHTS

	Testes g.	Spleen g.	Kidney g.	Adrenal mg.	Heart g.	Liver g.
I 50 r/day; No Drug	1.131	.544	2.042	.0404	1.275	10.6
II 50 r/day; 50 mg/Day	1.101	.505	2.084	.0408	.962	10.4
III 50 r/day; 75 mg/Day	1.128	.475	2.222	.0500	.883	11.0
IV 50 r/day 100 mg/Day	1.118	.489	1.921	.0419	1.006	10.3
V No r; No Drug	3.402	.487	1.957	.459	.916	10.1
VI No r; 50 mg/Day	3.394	.538	1.892	.0423	.878	9.1
VII No r; 75 mg/Day	3.135	.564	2.056	.0464	.844	9.0
VIII No r; 100 mg/Day	3.258	.577	2.061	.0443	.927	9.3

[1] The amount of drug indicated represents that amount of WR-361 made available to each rat. The average amount of drug actually consumed is shown in Table 2.

FIG. 1. BODY WEIGHT

Legend:
50 r/day - No Drug △——△
50 r/day - 50 mg/day o——o
50 r/day - 75 mg/day □——□
50 r/day - 100 mg/day ✗——✗
No r - No Drug △---△
No r - 50 mg/day o---o
No r - 75 mg/day □---□
No r - 100 mg/day ✗---✗

WITHDRAWAL OF DRUG AND IRRADIATION

BODY WEIGHT (GRAMS)

WEEKS

FIG. 2. LEUCOCYTE COUNT

19.2

WITHDRAWAL OF DRUG AND IRRADIATION

AVERAGE LEUCOCYTE COUNT (Thousands per Cu. mm. of Blood)

WEEKS

Legend:
△——△ 50 r/day - No Drug
o——o 50 r/day - 50 mg/day
□——□ 50 r/day - 75 mg/day
✗——✗ 50 r/day - 100 mg/day
△---△ No r - No Drug
o---o No r - 50 mg/day
□---□ No r - 75 mg/day
✗---✗ No r -100 mg/day

FIG. 3. LYMPHOCYTE AND NEUTROPHIL COUNTS
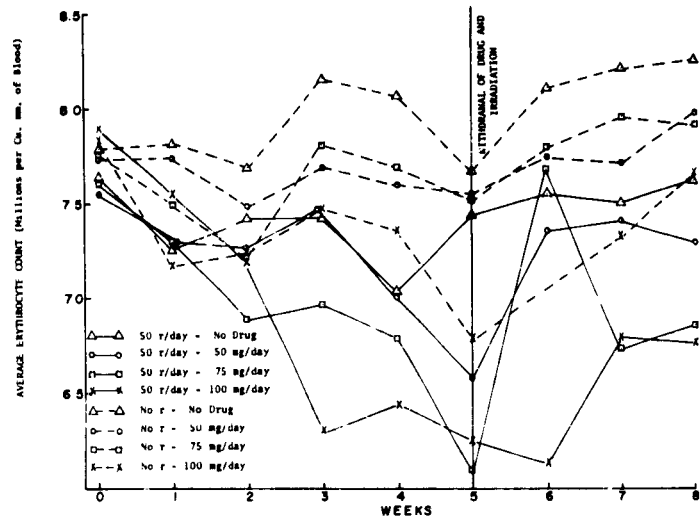
FIG. 4. ERYTHROCYTE COUNT
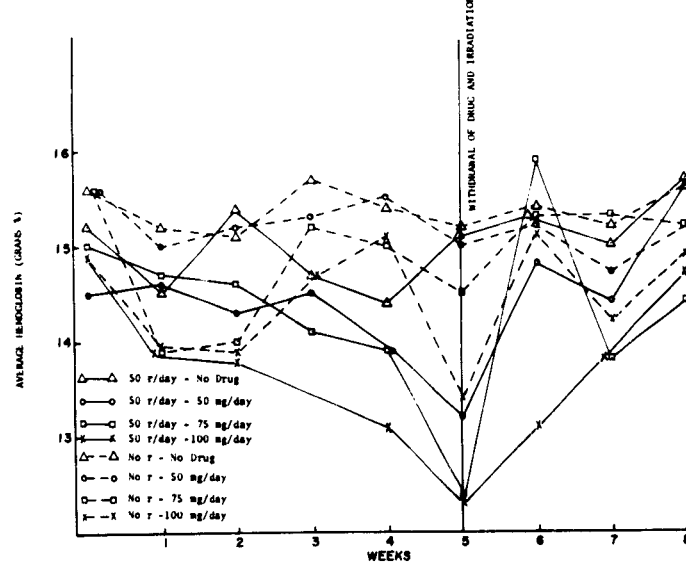
FIG. 5. HEMOGLOBIN VALUES
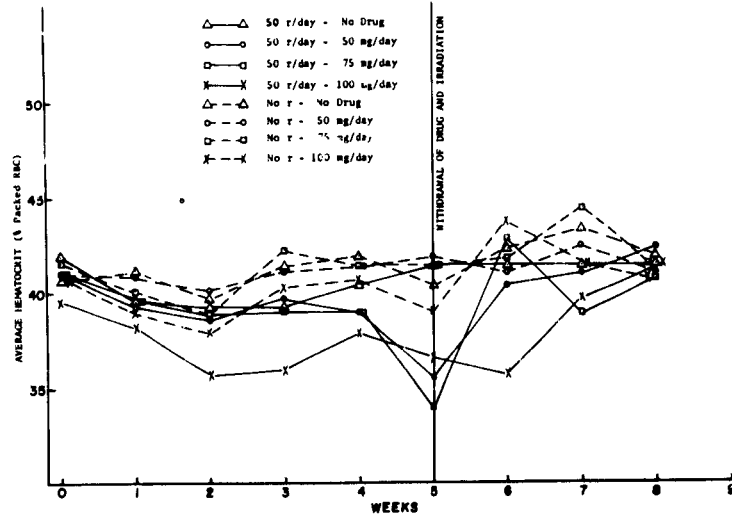
FIG. 6. HEMATOCRIT VALUES

FIG. 7. PERCENT SURVIVAL

Group I - 50 r/day; No Drug

Group V - No r; No Drug

Group II - 50 r/day; 50 mg/day

Group VI - No r; 50 mg/day

Group III - 50 r/day; 75 mg/day

Group VII - No r; 75 mg/day

Group IV - 50 r/day; 100 mg/day

Group VIII - No r; 100 mg/day

WEEKS

WEEKS

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